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Chromosomal variation in Agromyzidae (Diptera)

VI. Comparative chromosome studies

Lund 1977

Hereditas 84: 177—212 (1976)

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BLOCK, K. 1976. Chromosomal variation in Agromyzidae (Diptera). VI. Comparative chromosome studies. — *Hereditas 84*: 177—212. Lund, Sweden. ISSN 0018-0661. Received October 21, 1976

The polytene chromosomes of nine species were investigated and chromosome maps are presented. Together with four earlier investigated species, two of which consist of two semispecies each, they are compared as to chromosomal relationships.

The basic chromosome number 2n = 12 has been reduced by one centric fusion in one semispecies and by three centric fusions in three species each. The chromosome number has increased through polyploidy in the parthenogenetic species *Phytomyza crassiseta* to 2n = 18. In species with 2n = 12, five rods and one dot occur in five species, six rods occur in four species (or semispecies) and one V and five rods occur in one species.

In polytene chromosome cells there are always five euchromatic major arms and (as a rule) one very small one. The relative lengths of chromosome arms are similar within genera. Intra-arm-rearrangements have resulted in a great dissimilarity among species as to band sequences. In two groups of species it has been possible to homologize the chromosomes and to estimate the number of breaks lying behind species differentiation. One of these groups included three and the other two species.

Chromosomal polymorphism occurred in five species. Structural rearrangements, fixed in heterozygous condition, occurred in the parthenogenetic species. Inversions, fixed in homozygous condition, (incipient species differentiation) occurred in two species. Chromosomal evolution seems to have been due to paracentric inversions, changes in single bands and increase in the amount of constitutive heterochromatin.

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The family Agromyzidae is interesting from several aspects. As all its members are leaf miners they are as a rule more or less dependent on a certain host plant. In many cases, a species can be identified by studies of mines and larvae, whereas adult flies are often difficult to identify on external morphology. The male genitalia are, however, very detailed structures, which make it possible to distinguish species similar in the external morphology. Studies of the polytene chromosomes could give information as to the value of different systems of classification and might also contribute to the solution of taxonomical problems.

Systematically, the family Agromyzidae seems to be related to Drosophilidae, although the former is relatively isolated. Ecologically, however, there are great differences between the two families due to the close dependence on a certain host plant species, genus or family, which is typical for members of the family Agromyzidae. It would be interesting to know, how this ecological dependence influences chromosomal relationships. Host plant specificity might also exert influence both on species differentiation and on polymorphism.

Unfortunately, most members of Agromyzidae have rather unfavourable polytene chromosomes. This is probably one reason why only a few species have been cytologically investigated. *Liriomyza urophorina* MIK. was investigated by MAINX (1951) and by MAINX et al. (1956). This species showed chromosomal polymorphism for six paracentric inversions. STALKER (1954) compared a number of species belonging to different dipteran families as to the occurrence of polytene chromosomes in the nurse cells of the ovaries. More or less pronounced tendencies to polyteny were found in several species, but only a few showed useful polytene chromosomes. One of the most favourable ones was an Agromyzidae, *Cerodontha denticornis*. DA CUNHA (unpubl.) has investigated the chromosomes of *Melanagromyza tropicalis*. This is a species belonging to the subfamily Agromyzinae in contrast to all other cytologically investigated species, which belong to Phytomyzinae. In my own earlier work, four species are included. *Phytomyza abdominalis* ZETT. had no polymorphism but is split into two semispecies differing in a number of fixed inversions (BLOCK 1969a, 1975a). Natural hybridization occurs and the hybrids are at least to

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Table 1. Agromyzidae species, host plants and localities

Species	Host plants in literature	Kind of specifi-city[1]	Present host plants	Present localities
Phytomyza abdominalis ZETT.	*Anemone hepatica* L.	Mon. 1	*Anemone hepatica* L.	Several [2,3]
Phytomyza crassiseta ZETT.	*Veronica* L., several species	Mon. 3	*Veronica* L., several species	Several[4]
Phytomyza ilicis CURTIS	*Ilex aquifolium* L.	Mon. 1	*Ilex aquifolium* L.	Lund, Copenhagen, Hamburg
Phytomyza primulae ROB.-DESV.	*Primula* L., several species	Mon. 3	*Primula veris* L., *Primula elatior*	Lund, localities on Öland
Amauromyza (*Trilobomyza*) *flavifrons* (MEIGEN)	Caryophyllaceae, Chenopodiaceae	Olig. 2	Caryophyllaceae, several genera	Several[5]
(*Trilobomyza*) *verbasci* (BOUCHÉ)	*Verbascum*, *Scrophularia*	Olig. 1	*Verbascum thapsus*, *V. nigrum*	Kävlinge, Åkarp (Skåne)
Cerodontha (*Butomomyza*) *eucaricis* NOWAKOWSKI	*Carex* L., several species	Mon. 3	*Carex hirta* L., *C. pseudocyperus*	Several[6]
(*Butomomyza*) *pseuderrans* (HENDEL)	*Carex hirta* L.	Mon. 1	*Carex hirta* L.	Silvåkra, Sillaröd (Skåne), loc. on Öland
(*Butomomyza*) *vignae* NOWAKOWSKI	*Carex* L., several species	Mon. 3	*Carex remota* L.	Localities on Öland
(*Butomomyza*) *angulata* (LOEW)	*Carex* L., several species	Mon. 3	*Carex hirta* L.	Silvåkra, Sillar., Osbyholm (Sk.), loc. on Öland
(*Butomomyza*) *scirpi* (KARL)	*Scirpus silvaticus*, *S. maritimus*	Mon. 2	*Scirpus silvaticus*	Silvåkra, Sösdala (Skåne)
(*Dizygomyza*) *ireos* (GOUREAU)	*Iris pseudacorus* L., *Iris sibirica*	Mon. 2	*Iris pseudacorus* L.	Silvåkra (Skåne), Torslunda (Öland)
(*Dizygomyza*) *spinata* (GROSCHKE)	*Carex pilosa* SCOP, *C. silvatica*	Mon. 2	*Carex pilosa* SCOP	Hungary, Yugoslavia

[1] According to the terms used by HERING (1951)
Abbreviations: Mon. 1 = monophagy, first degree
Mon. 2 = monophagy, second degree
Mon. 3 = monophagy, third degree
Olig. 1 = systematic oligophagy, first degree
Olig. 2 = systematic oligophagy, second degree

[2] BLOCK 1969a
[3] BLOCK 1975a
[4] BLOCK 1969b
[5] BLOCK 1975b
[6] BLOCK 1974

some extent fertile. *Phytomyza crassiseta* ZETT. is a parthenogenetic species (BLOCK 1969b). Three different types of strains could be found in this species: sexual diploids, parthenogenetic diploids and parthenogenetic triploids. The triploids contained nine different inversions and two changes in single bands. These rearrangements occurred and were combined in four strains or clones. *Cerodontha* (*Butomomyza*) *eucaricis* NOWAKOWSKI showed a similar incipient species differentiation as *Phytomyza abdominalis* but contained two more or less polymorphic semispecies (BLOCK 1974). *Amauromyza* (*Trilobomyza*) *flavifrons* MEIGEN showed polymorphism in double and single inversions as well as changes in single bands (BLOCK 1975b).

The present investigation, which includes nine more species, all belonging to Phytomyzinae, is an attempt to tie up the previous information and give a general review of the type of chromosomal changes that might have been at work during evolution. The total number of species is probably extremely large. Therefore, no definite conclusions can be drawn concerning chromosomal evolution. Information might be obtained, however, as to the connection between inter- and intraspecific variation.

Animal material

Table 1 exhibits the scientific names of the thirteen species investigated by me together with information on which plants they were collected from and some short information on locality. In addition, information is given concerning which plants the species were collected from earlier. This is important for establishing a characterization of the degree of specificity. According to HERING (1951) monophagy means that the host range of the parasite is restricted to one plant genus. In this sense, most of the species cytologically investigated are monophagous. Among

these monophagous species, however, only three are species-specific, i.e. display monophagy of the first degree. These three species are *Phytomyza abdominalis*, *Phytomyza ilicis* and *Cerodontha* (*Butomomyza*) *pseuderrans*. Three species are restricted to a section within a plant genus e.g. they display monophagy of the second degree, namely *Cerodontha* (*Butomomyza*) *scirpi*, *Cerodontha* (*Dizygomyza*) *ireos* and *C.* (*D.*) *spinata*. The most common kind of specificity seems to be monophagy of the third degree. Such species are restricted to a plant genus but can occur in all species within this genus. In the present material, however, the three *Butomomyza*-species are collected from only one or two species each. Only two of the thirteen species occur in more than one genus. *Amauromyza* (*Trilobomyza*) *verbasci* is recorded from both *Verbascum* and *Scrophularia*, and *A.* (*T.*) *flavifrons* from both Caryophyllaceae and Chenopodiaceae. According to Hering's terminology, *Trilobomyza verbasci* should be regarded as *systematic oligophagous* of the first degree and *Trilobomyza flavifrons* as *systematic oligophagous* of the second degree.

All material was collected directly from the natural localities at the larval stage except for the parthenogenetic species *Phytomyza crassiseta*. In this case some material was collected at the pupal stage. The chromosomes were then studied in the descendants of the naturally collected individuals.

In the genus *Phytomyza*, cells from the salivary glands contained the most favourable polytene chromosomes. In the genus *Cerodontha*, on the other hand, salivary gland chromosomes were small and the bands rather indistinct. Gastric coeca cells, however, had large polytene chromosomes with distinct bands. In *Amauromyza* (*Trilobomyza*) *verbasci* both tissues contained cells with analyzable polytene chromosomes, but those of the gastric coeca cells were much larger. In *A.* (*T.*) *flavifrons* both types of cells were of equally good quality.

Methods

Most preparations were made with the orcein squash method without previous fixation. When much material was collected at the same time, larvae were fixed in alcohol—acetic acid 3:1 and after fixation stained either with the Feulgen method, in Gomory's hematoxylin according to Melander and Wingstrand (1953) or in Snow's carmine (Snow 1963). When any of the last three methods was used, entire larvae were punctured, fixed and stained. All four methods resulted in the same banding pattern and relative lengths of the chromosomes. The absolute lengths were, however, much larger in cells stained with orcein. Most likely, this is due to a swelling effect of acetic acid compared with fixation in alcohol—acetic acid. Therefore, absolute lengths are not itemized in the present paper. Preparations have been made permanent with the dry-ice method (Conger and Fairchild 1953). As a rule, however, microphotographs were made from semi-permanent preparations, which were only sealed with Krönig's cement.

Microphotographs were made on the scientia film 23 D 56 with a magnification of 1100×. For measurements, copies with 2200× magnification were used. In most cases, 10 chromosome complements from each species were measured with a "map measurer" and the relative length of each chromosome was calculated. After that, a number of regions within each arm were identified and the length of each region was calculated. Finally, each chromosome was drawn region for region from microphotographs. The mapping was fulfilled by dividing the whole chromosome complement into 100 regions. Each chromosome was thus divided into a number of regions in proportion to its relative length. The length of each region was modified in such a way that it always began with a distinct band. Whether the chromosomes were metacentric or telocentric, all chromosome arms were drawn with the centromeres to the left. Centromeric heterochromatin of the loose beta-type was not drawn in the map, as this type of heterochromatin displays variation from cell to cell.

Mitotic chromosomes were studied in neuroblast cells or — in some cases — in gonads. They were stained with acetic orcein. No measurements were made on mitotic chromosomes.

Results

1. Description of the chromosomes

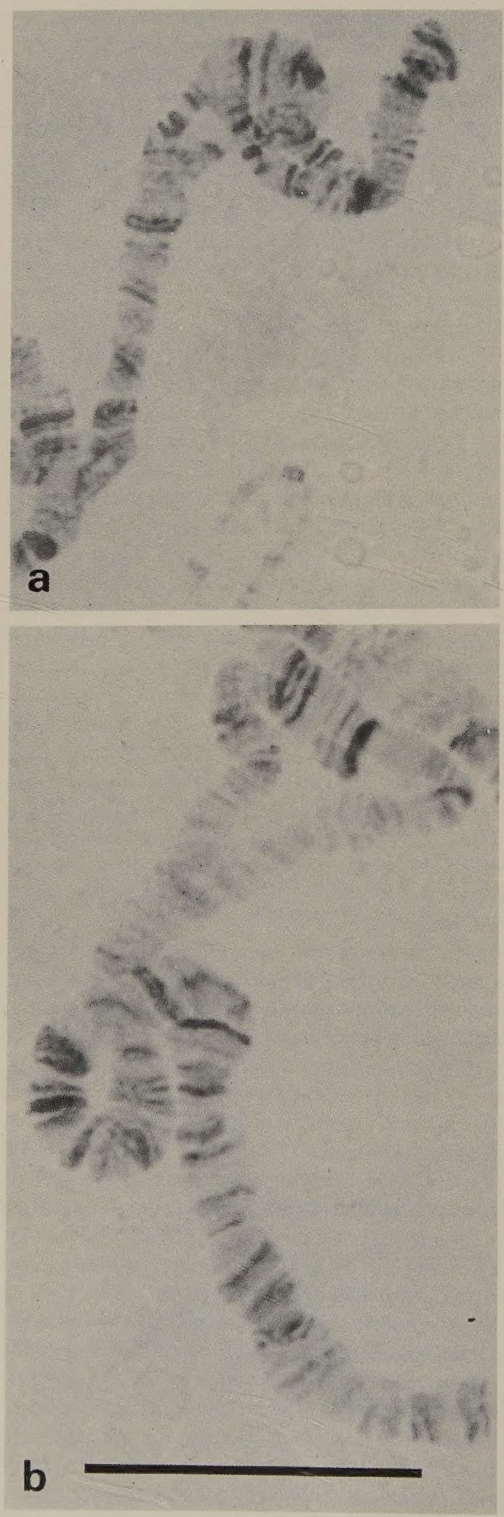
A. *Genus Phytomyza* Fallén

Phytomyza abdominalis Zetterstedt

The chromosomes of this species have been described earlier (Block 1969a).

Phytomyza crassiseta Zetterstedt

The chromosomes of this species have also been described earlier (Block 1969b). In this species parthenogenetic reproduction occurs, but both sexual diploids, parthenogenetic diploids and parthenogenetic triploids are observed. Nine inversions, one



duplication and one deletion have been found in the triploids. Since the previous publication, two heterozygous inversions have been observed in the diploids. These inversions are illustrated in Fig. 1. One of them has an approximate position from region 20 up to region 23 in chromosome 1 (Fig. 1a). For the second inversion, illustrated in Fig. 1b, the position is from region 80 up to the end of region 82 in the X chromosome (chromosome 4). These regions refer to the chromosome maps from 1969. The two heterozygous inversions did not occur in the same individuals.

Phytomyza ilicis CURTIS

Mitotic cells from somatic tissues contain five rod-shaped and one dot-like pair. In gonial cells from testes and ovaries there are in addition a variable number of chromosomes, which are a little larger than the dot-like pair. These germ-line limited chromosomes displayed variation in number even within individuals.

The polytene chromosomes are illustrated in the chromosome maps, Fig. 2. All chromosomes are telocentric except perhaps chromosome 2, containing heterochromatin, which might be centromeric heterochromatin. In this case, it is not situated at the chromosomal end. A tendency towards a common chromocentre can be observed but it is not very obvious. All chromosomes can be easily identified.

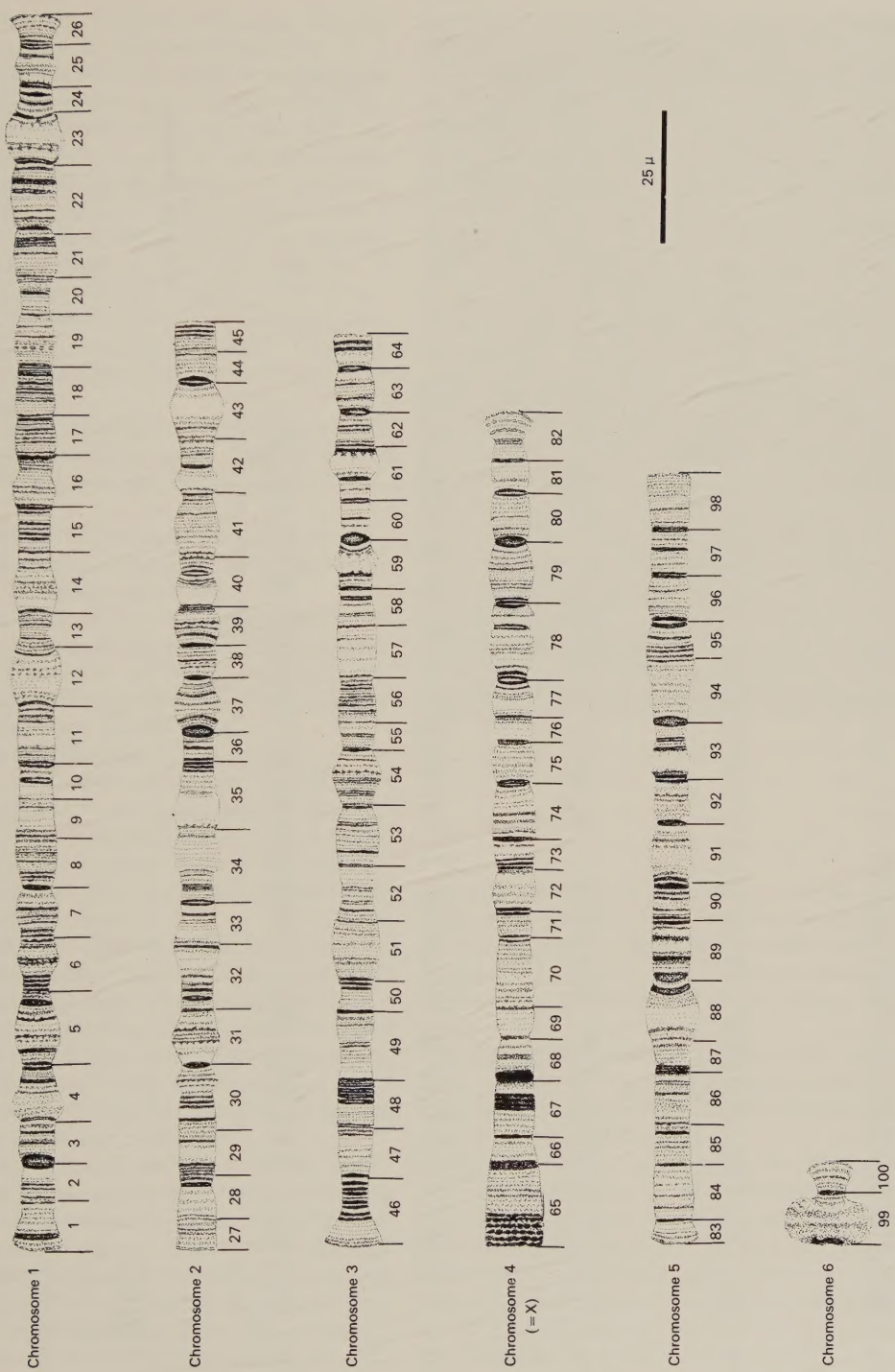
Chromosome 1. Region 1—2 and the broad band between regions 2 and 3 are good characteristics for this chromosome. A large puff in region 23 is as a rule present. When the larva is exposed to low temperatures, this puff can reach a considerable size. It is a useful landmark.

Chromosome 2. Between regions 28 and 29 there are several dark bands. The chromosome is often broken in this region, which often sticks together with the common chromocentre. This indicates the presence of heterochromatin. The centromere might be situated in connection with this region. Another landmark is region 43, which consists of a puff and ends with a thin but conspicuous band.

Chromosome 3. This chromosome is characterized by one puff in region 59 and one in 61. Both show a characteristic shape.

Chromosome 4. This chromosome represents the X chromosome. It displays a different morphology in males and females. In the females the two paired X chromosomes contain distinct bands following the

Fig. 1a and b. *Phytomyza crassiseta*. Heterozygous inversions in the diploid; a: inversion in chromosome 1; b: inversion in the X chromosome (chromosome 4). Scale: 25 μ.


Chromosome 1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Chromosome 2
27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Chromosome 3
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64
Chromosome 4
(=X)
65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82
Chromosome 5
83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98
Chromosome 6
99 100
25 µ

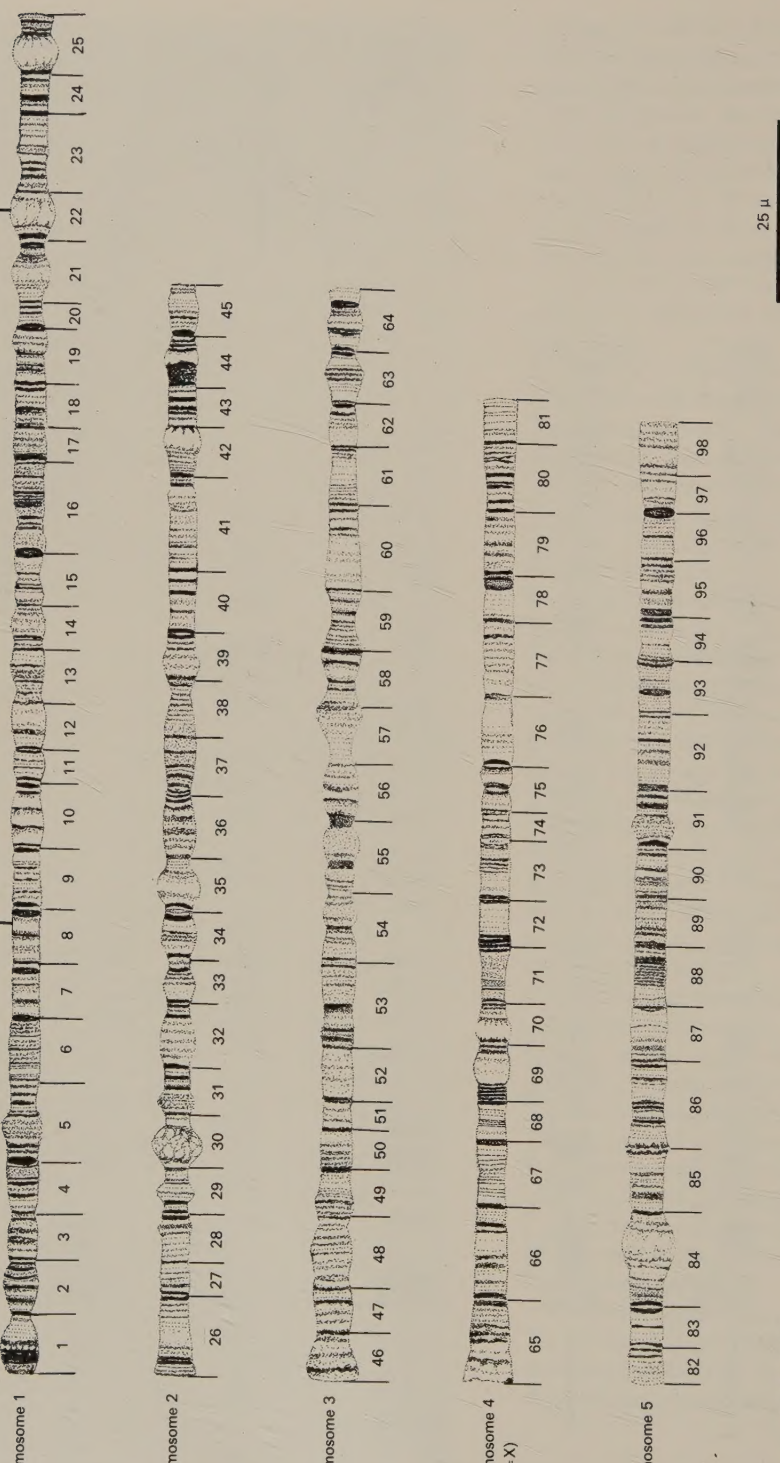


Fig. 3. *Phytomyza primulae*. Chromosome maps. Description in the text.

pattern illustrated in the chromosome map. In males there is only one X chromosome and the Y is entirely heterochromatic. The single X chromosome of the males contains only a few conspicuous bands, its length being reduced to little more than half that of the females. Both length and structure display variation from individual to individual and even from cell to cell. This type of allocycly is typical for the X chromosome of several species and the phenomenon will be discussed later on. In both sexes the X chromosome is the only one which contains centromeric heterochromatin of the loose beta-type. This heterochromatin is a useful landmark. There are also typical dark bands near the heterochromatin in regions 65 and 67.

Chromosome 5. The regions near the centromere are free from conspicuous bands. Regions 89—90 show several dark but thin bands, situated close together.

Chromosome 6. This dot-like chromosome is identified by its small size. There is a large puff in region 99, visible in all salivary gland cells.

In this species no chromosomal polymorphism has been observed although more than 400 individuals have been investigated. All these individuals are, however, collected from gardens. They were collected in Lund, Copenhagen and Hamburg.

Phytomyza primulae ROBINEAU-DESVOIDY

In mitotic metaphase there are five rods and one dot. In the polytene chromosomes there are five long arms and one very small chromosome, which might be metacentric. The polytene chromosomes are illustrated in the chromosome maps, Fig. 3. The following landmarks are most useful.

Chromosome 1. This chromosome contains a cluster of alfa-heterochromatin near the centromere (region 1) and a puff near the telomere (region 25).

Chromosome 2. Regions 43—45, which form the terminal segment, display some very deeply stained bands and a small puff in the middle of region 44.

Chromosome 3. Region 64, the terminal region, has two dark bands near each other.

Chromosome 4. This is the X chromosome, which is recognized by its centromeric heterochromatin. The nucleolus is organized in or in the neighbourhood of this heterochromatic region. The same allocycly as in the X chromosome of *Phytomyza ilicis* may be observed, but it is not so generally expressed. Thus, the single X chromosome may show distinct bands.

Chromosome 5. In the terminal regions, 96—98, a very broad, dark band is visible between the regions 96 and 97. The surrounding bands are only weakly stained.

Chromosome 6. This is the smallest, dot-like chromosome. It contains a large heterochromatic block in the middle of the chromosome. Probably the centromere is situated in connection with this region. It is the only dot-like chromosome that appears to be metacentric in all the species investigated.

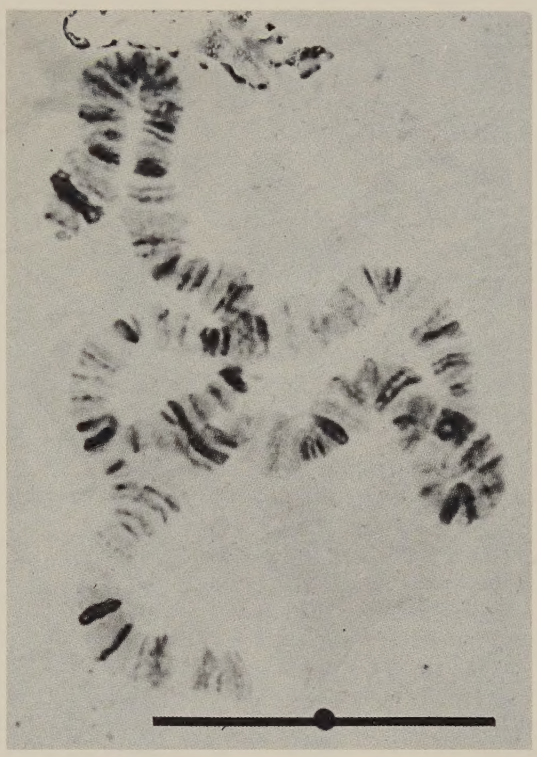


Fig. 4. *Phytomyza primulae*. Heterozygous inversion in chromosome 1. Scale: 25 μ.

In *Phytomyza primulae* a large inversion, reaching from region 8 to region 22 in chromosome 1 has been found in one individual in heterozygous condition. It is illustrated in Fig. 4.

These four species belonging to the genus *Phytomyza* show similarities in the general appearance of their polytene chromosomes. In all four species there are five long arms and one very small chromosome. The chromosome arms represent separate chromosomes except in *Phytomyza abdominalis* where the X chromosome and the smallest chromosome have fused in one of the two semispecies. In *Phytomyza primulae* the smallest chromosome might have become metacentric owing to a pericentric inversion. The X chromosome is number 4, perhaps with the exception of *Phytomyza abdominalis*, where it is

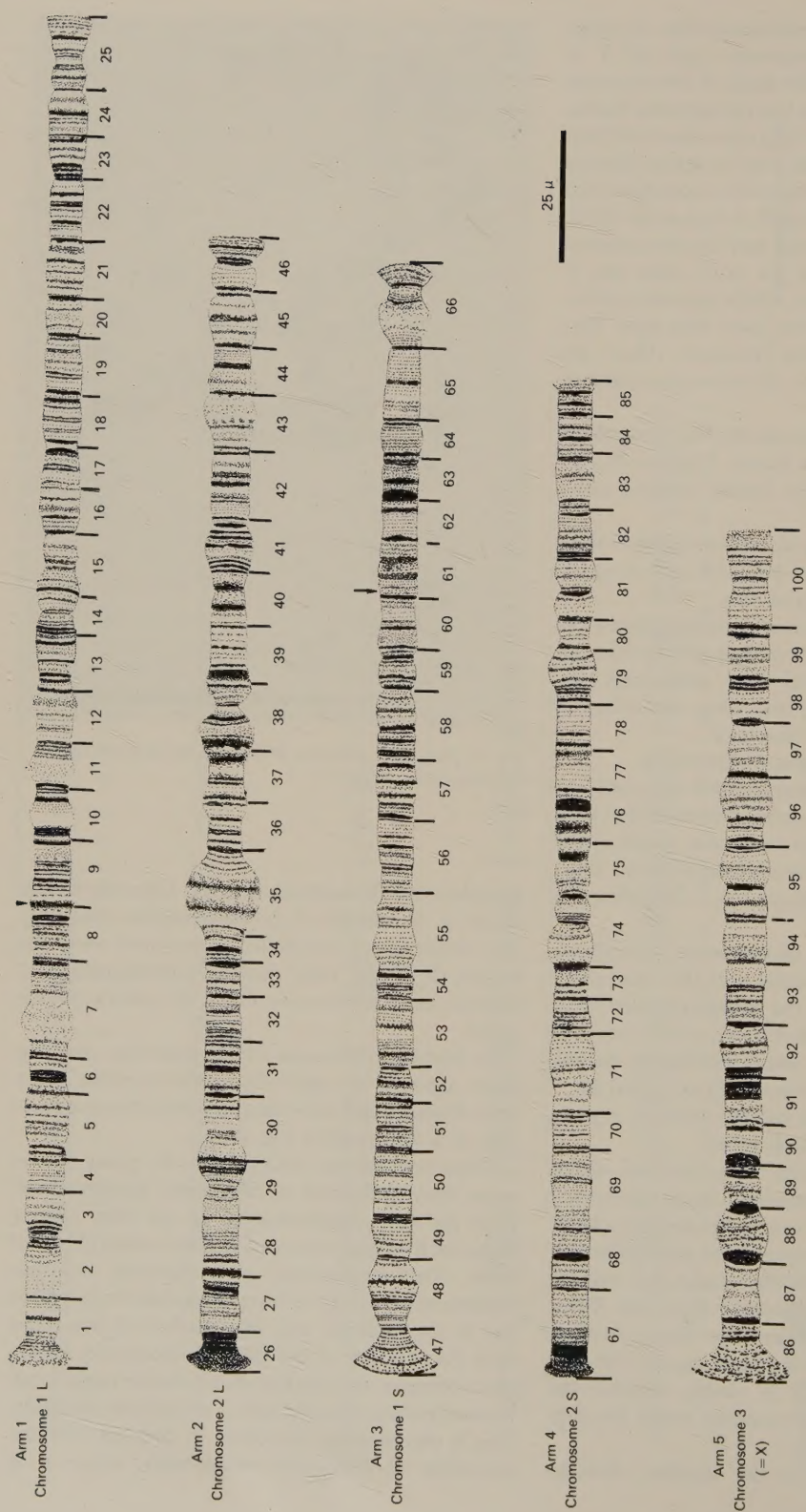


Fig. 5. *Amauromyza* (*Trilobomyza*) *verbasci*. Chromosome maps. Description in the text. Arrows indicate points, where the chromosomes are regularly broken by the squash.

number 2 or 3. Here, however, chromosomes 2, 3 and 4 have similar lengths. In all four species the X chromosome is the only one that contains centromeric heterochromatin of the loose beta-type. The relative lengths are also similar. This will be discussed later on.

In contrast to these similarities in the general appearance, band sequences are entirely different and no segments can with certainty be homologized from species to species.

B. *Genus Amauromyza* HENDEL

Subgenus *Trilobomyza* HENDEL

Amauromyza (*Trilobomyza*) *flavifrons* (MEIGEN)

In mitotic metaphase there are two pairs of metacentric and one pair of submetacentric chromosomes. The polytene chromosomes were described earlier (BLOCK 1975b). Chromosomal polymorphism occurs.

Amauromyza (*Trilobomyza*) *verbasci* (BOUCHÉ)

The mitotic chromosome complement is similar to that of *A.* (*T.*) *flavifrons* e.g. 2 metacentric and one submetacentric pair.

In polytene chromosome cells there are five long strands, which represent five chromosome arms. No dot-like chromosome is visible, but there might be a completely heterochromatic one. Polytene chromosomes are of more superior quality in gastric coeca cells than in salivary gland cells. They are possible to analyse, however, in both types of cell. Also, in the intestine, it is possible to identify all chromosome arms, but the bands are less conspicuous. In both salivary gland cells and in gastric coeca cells the five arms become completely separate due to the squash. Thus, it is not possible to decide from these tissues which arms belong to the same chromosome. In the intestine, however, three chromosomes are visible. As in *Trilobomyza flavifrons*, there are certain points where a chromosome is regularly broken by the squash. These points are different in different tissues. It thus often appears to be six arms. The polytene chromosomes are illustrated in the chromosome maps, Fig. 5, and are identified in the following way:

Arm 1. (Chromosome 1 L). Region 1 contains centromeric heterochromatin of the loose beta-type and very few dark bands. In the border between regions 8 and 9 the chromosome is regularly broken in the salivary gland cells. Regions 10 and 23 are good landmarks. The terminal end of the chromosome is similar to that of *Trilobomyza flavifrons*.

Arm 2. (Chromosome 2 L). This arm contains no heterochromatin of the loose beta-type. There are some dark bands near the centromere, which might represent centromeric heterochromatin of the compact alfa-type. This arm also contains a large puff in region 35. Region 39 contains a broad band followed by a thinner one. The end segment, region 46, is also characteristic. In this arm too, there are terminal segments possible to recognize from *Trilobomyza flavifrons*.

Arm 3. (Chromosome 1 S). As in the long arm of chromosome 1 there is a large block of centromeric heterochromatin of the loose beta-type, situated here in region 47. At the beginning of region 61 there is a point where the chromosome is regularly broken in the gastric coeca cells. In region 66, there is a puff in the gastric coeca cells.

Arm 4. (Chromosome 2 S). There are dark bands near the centromere, probably representing centromeric heterochromatin of the compact alfa-type. After these bands follows a segment with less deeply stained bands and in region 68 there is a thin but very distinct band. Region 76 is characteristic, and similar to region 79 in *Trilobomyza flavifrons*. This region is a landmark for arm 4 in this species too. The same is the case for the terminal segments 84 and 85, which are similar to segments 83 and 84 in *Trilobomyza flavifrons*.

Arm 5. (Chromosome 3 = X). This chromosome, like arms 1 and 3 contains loose heterochromatin. Region 88 begins and ends with deeply stained bands and the chromosome is slightly broadened. In both regions 89 and 90, there are several deeply stained bands. The distal segment, region 100, contains no dark bands.

The small dot-like chromosome, which is visible in most species, seems to be absent here. Either it has been translocated to one of the large chromosomes or it has become heterochromatic. The latter possibility seems to be more likely, because a dot-like chromosome — without distinct bands — is visible in some cells of *Trilobomyza flavifrons* (BLOCK 1975b).

The two species of the subgenus *Trilobomyza* show some similarities in chromosomal morphology. First, the general appearance is similar: both species have five arms, representing three chromosomes, the X chromosome is number 5, and the small dot-like chromosome has disappeared or has become heterochromatic. Secondly, both species have large clusters of loose heterochromatin in contrast to the four *Phytomyza*-species, which have the loose heterochromatin restricted to the X chromosome. Thirdly, there are great similarities in the band sequences, and in all five arms homologous segments can be found. Finally, the relative lengths of the homologous

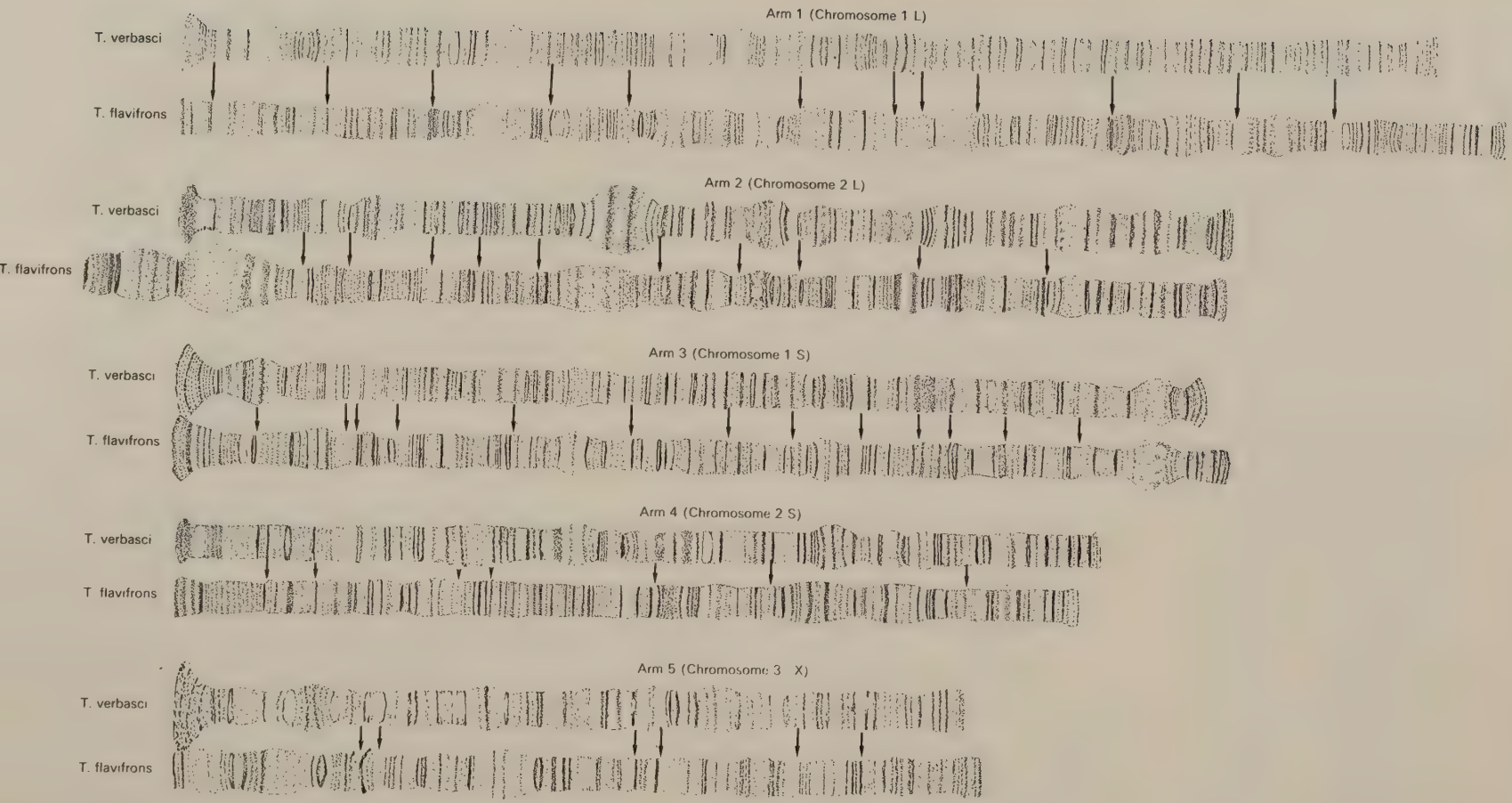


Fig. 6. Comparison of the chromosome maps of *Trilobomyza flavifrons* and *T. verbasci*. The chromosome maps of *T. verbasci* are left uncut, whereas those of *T. flavifrons* are cut into pieces. Arrows indicate break points, which are necessary for as close resemblance as possible.

arms are similar. The last aspect will be discussed later on.

In order to get more detailed information on the structural differences between the two species, their chromosome maps were compared in the following way: First, copies have been made of the chromosome maps of the two species. The maps of *Trilobomyza flavifrons* were cut into pieces, and pieced as well as possible on to segments of the homologous segments of *Trilobomyza verbasci*. By this technique, it was to some extent possible to reconstruct the breaks, which could have occurred during differentiation of the two species. The result of this comparison is illustrated in Fig. 6. Although there are many limitations in this method of comparison, a few comments could be made. At least 56 breaks are involved in the differentiation of the two species. There are many small regions, which do not display any similarities in the homologous chromosome of the other species. None of these segments could be recognized in other chromosomes either. Thus, they cannot be explained by translocations or insertions.

A general observation is that *Trilobomyza verbasci* has more intensively stained bands than *T. flavifrons*, which could mean that at least some of the changes are duplications within single bands.

C. *Genus Cerodontha* RONDANI

Subgenus *Butomomyza* NOWAKOWSKI

Cerodontha (*Butomomyza*) *eucaricis* NOWAKOWSKI

The mitotic chromosome complement consists of one very large metacentric pair and five smaller telocentric pairs.

The polytene chromosome complement consists of five long arms and one dot-like chromosome. The polytene chromosomes have been described earlier (BLOCK 1974) together with chromosomal polymorphism and incipient species differentiation.

Cerodontha (*Butomomyza*) *pseuderrans* (HENDEL)

The mitotic chromosome complement consists of five large telocentric pairs and one dot-like pair, which is different from the complement of *Butomomyza eucaricis*.

The polytene chromosomes are illustrated in the chromosome maps, Fig. 7, and are characterized in the following way.

Chromosome 1. The dark band at the end of region 21 and the thin band at the beginning of region 22 form together a good landmark for this chromosome.

Chromosome 2. There is a large puff in region 42, followed by a more narrow segment in region 43. A double band at the border between regions 44 and 45 is another landmark. The end of this chromosome lacks distinct bands. All these segments are similar to regions 44—47 in *Butomomyza eucaricis*.

Chromosome 3. This chromosome is the easiest one to identify, as it contains a remarkable constriction surrounded by characteristic bands with a more or less circular orientation (regions 57—58). The two bands at the end of region 62 and the beginning of region 63 are also useful landmarks. These two landmarks are also observed in *Butomomyza eucaricis*, (regions 55—56 and 64—65, resp.).

Chromosome 4. The terminal segments, regions 81—83, are the best landmarks for this chromosome.

Chromosome 5. This is the X chromosome. In this species no allocycly like that of *Butomomyza eucaricis* has been observed. The bands are thus as a rule distinct in both sexes. The same landmarks are useful, namely the end segment, region 98. Region 94 is also a useful landmark and seems to be homologous to region 95 in *Butomomyza eucaricis*. It is, however, situated in another position.

Chromosome 6. This is the smallest dot-like chromosome. It lacks obvious landmarks and often there are no distinct bands at all.

No chromosomal polymorphism is observed in this species.

Cerodontha (*Butomomyza*) *vignae* NOWAKOWSKI

The mitotic chromosome complement consists of five telocentric and one dot-like pair as in *Butomomyza pseuderrans*.

The polytene chromosomes are illustrated in the chromosome maps, Fig. 8, and are characterized in the following way.

Chromosome 1. The dark bands in regions 21—22, observed in *Butomomyza pseuderrans*, occur here too. There are great similarities in the whole chromosome, especially in the regions 11—25.

Chromosome 2. The puff in region 42, the narrow segment in region 43 and the double bands in regions 44—45, observed in *Bytomomyza pseuderrans* occur in this species too. There are many similarities along the whole chromosome.

Chromosome 3. The characteristic constriction, which occurs in *Butomomyza eucaricis* and *Butomomyza pseuderrans* is found here too. It shows, however, a different position in different species and in this species it is situated in regions 58—59. The same characteristic bands near the end of the chromosome are also observed, in this species in the border between regions 63 and 64.

Chromosome 4. The terminal segments, regions 81—83, are the best landmarks. There are no obvious similarities with chromosome 4 in *Butomomyza pseuderrans*.

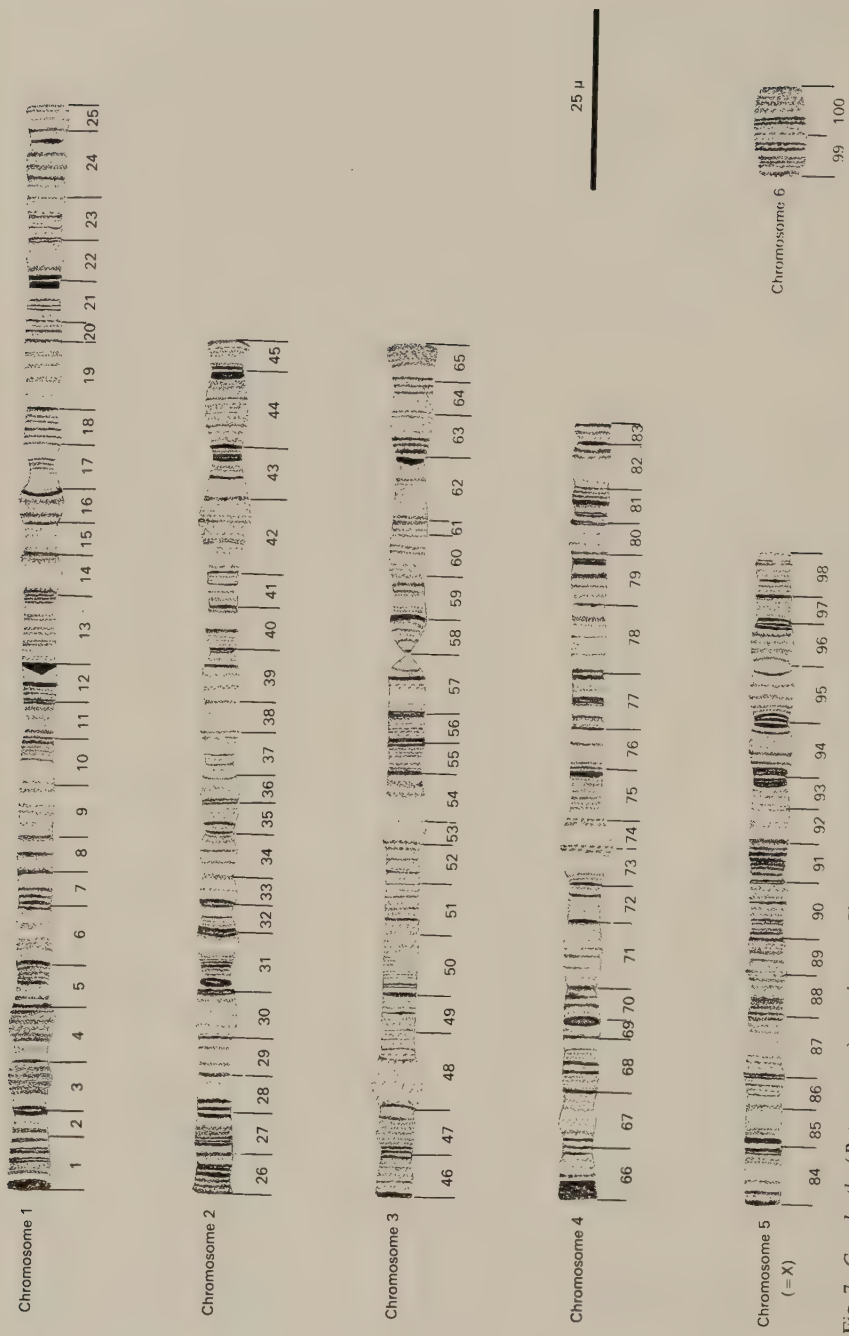


Fig. 7. *Cerodontha* (*Butomomyza*) *pseuderrans*. Chromosome maps. Description in the text.

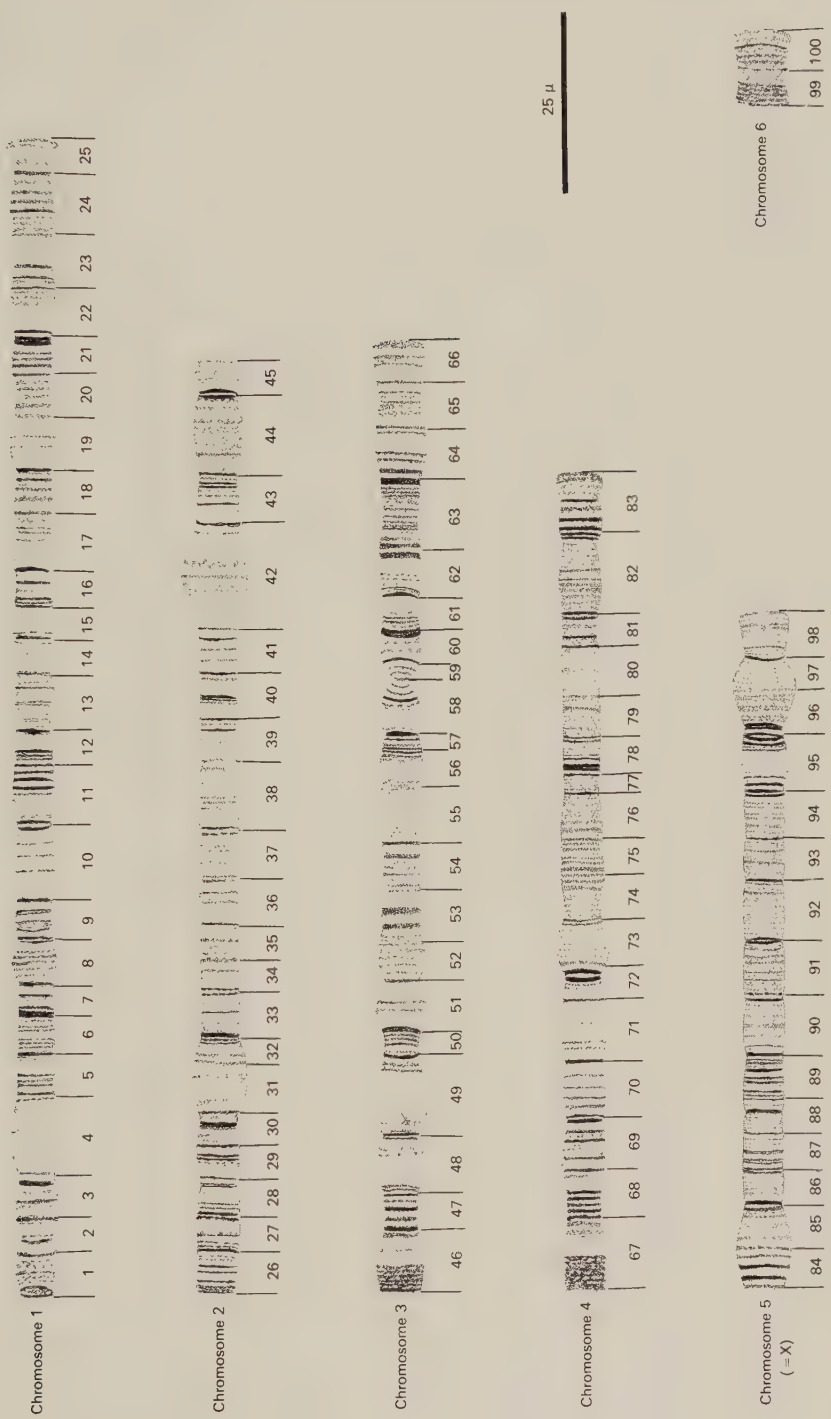
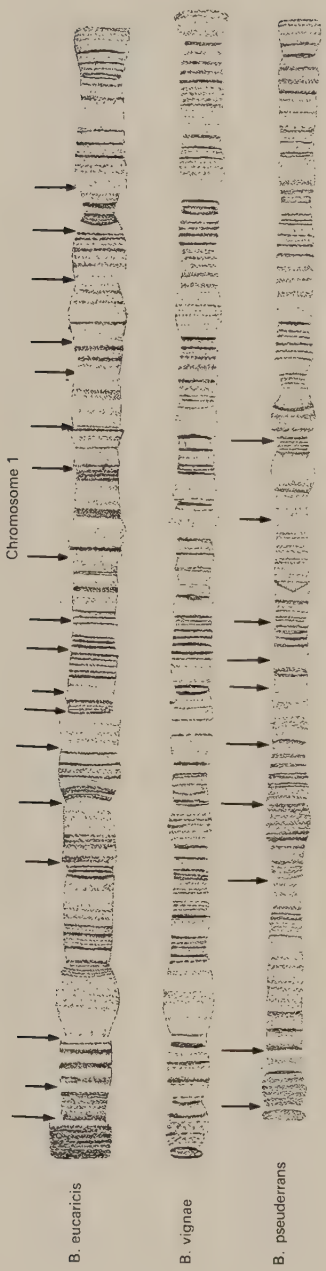
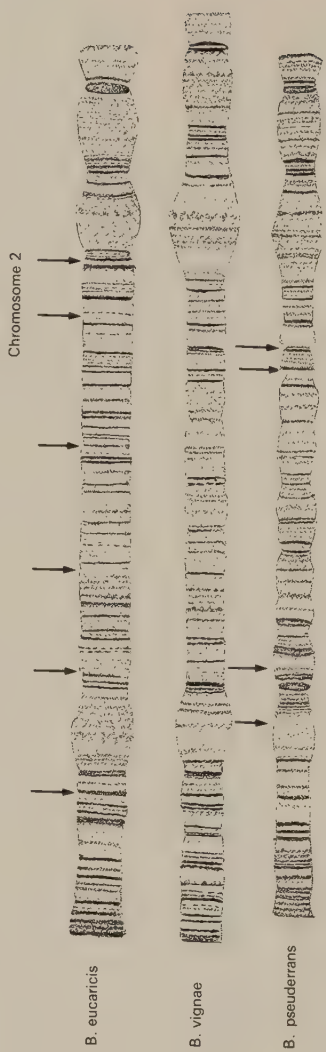


Fig. 8. *Cerodontha (Butomomyza) vignae*. Chromosome maps. Description in the text.


Chromosome 1
B. eucaricis
B. vignae
B. pseuderrans


Chromosome 2
B. eucaricis
B. vignae
B. pseuderrans

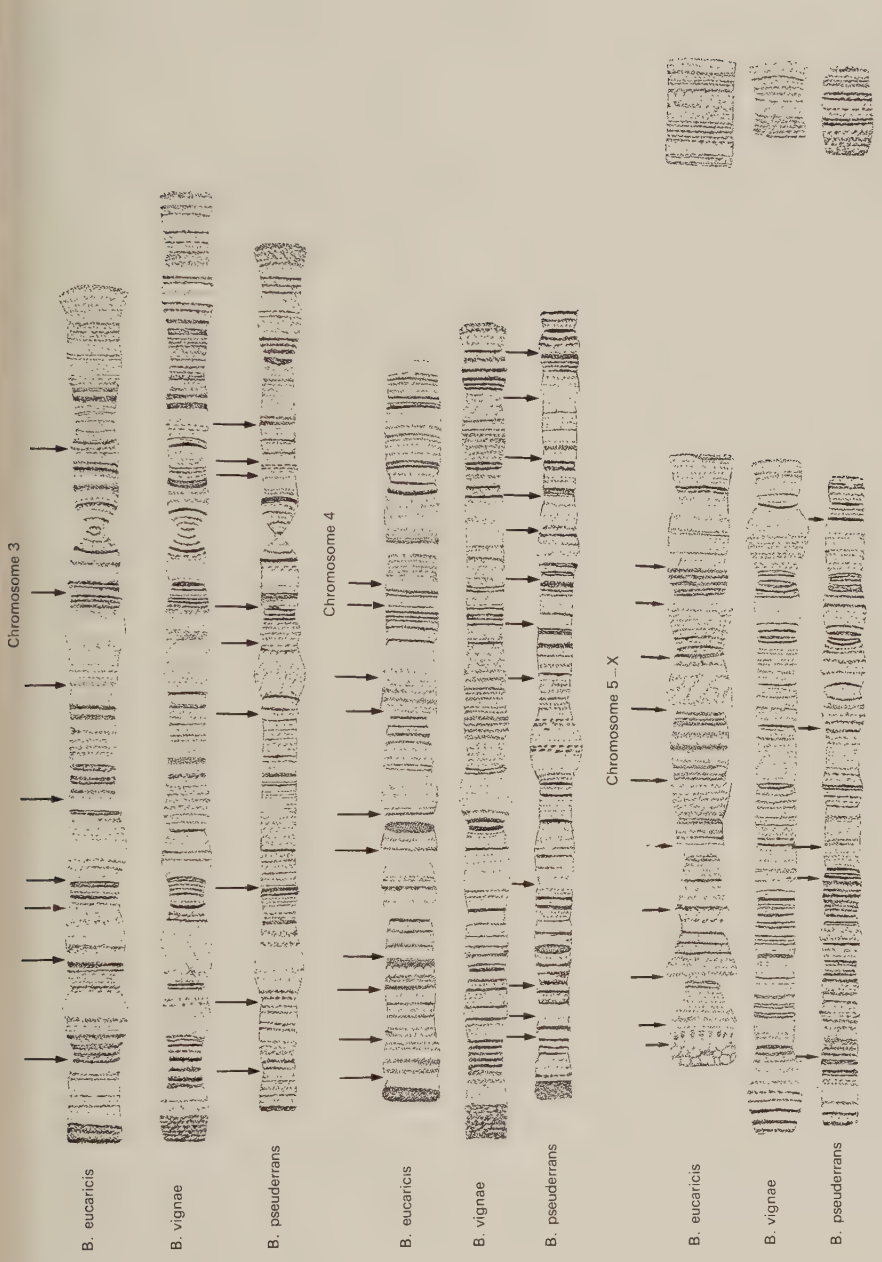


Fig. 9. Comparison of the chromosome maps of the three species *Butomomyza eucaricis*, *B. pseuderrans* and *B. vignae*. The maps of *B. vignae* are left uncut, whereas those of *B. eucaricis* and *B. pseuderrans* are cut into pieces. Arrows indicate break points, which are necessary for as close resemblance as possible.

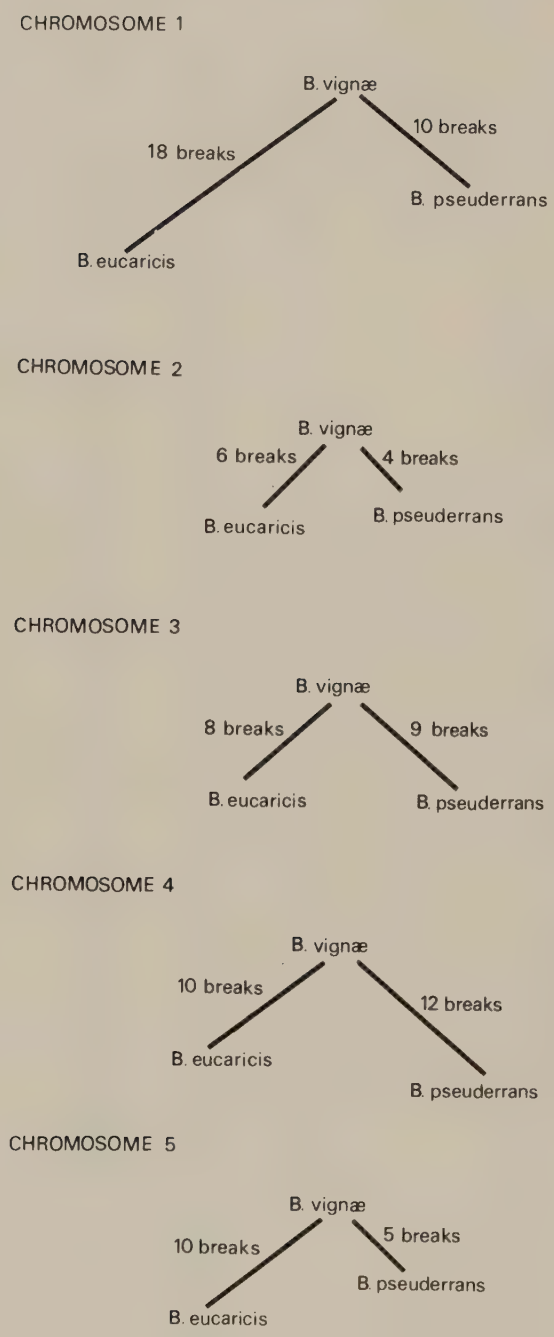


Fig. 10. Diagram representing the number of breaks differing the three species in the *pseuderrans* group of the genus *Cerodontha* (*Butomomyza*).

Chromosome 5. This is the X chromosome. As in *Butomomyza pseuderrans* the chromosome shows distinct bands in both sexes. The male single X chromosome may be a little thinner and a little more twisted. The terminal segments are most characteristic. Region 95, which is homologous to region 94 in *Butomomyza pseuderrans* begins and ends with clusters of dark bands. Regions 96—98 are very faintly stained with the exception of one dark band in the border between regions 97 and 98.

Chromosome 6. This small dot-like chromosome lacks obvious bands.

No chromosomal polymorphism has been observed.

The three *Butomomyza*-species referred to here belong to the same species group (Nowakowski 1972, 1973). The taxonomical classification is founded on morphological characters, both from larvae and adults. The cytological observations show that the morphological similarities are accompanied by chromosomal ones. All chromosomes contain homologous segments in the three species. The size relations also show a close resemblance. Chromosome 3 in *Butomomyza eucaricis* and *B. pseuderrans* is homologous to the one that is second in size in *B. vignae*. Size differences are, however, very small between chromosomes 2 and 3. It is also more convenient to have the same designation for homologous chromosomes. This is the reason why this chromosome is called chromosome 3 in *Butomomyza vignae* too.

Homologous chromosomes have been compared with the same technique as was used for the genus *Amauromyza* (*Trilobomyza*). *Butomomyza vignae* seems to have a morphology which is to some extent intermediate to that of the other two species. Therefore, the chromosome maps of *B. vignae* have been used as a standard, the maps of the other two species having been pieced on to it in order to get as close a resemblance as possible with the maps of *B. vignae*. The result is illustrated in Fig. 9.

In all, 52 breaks differentiate *B. eucaricis* and *B. vignae* and 40 breaks differentiate *B. pseuderrans* and *B. vignae* from each other. The resemblance after this reconstruction is also more complete between the maps of *B. pseuderrans* and *B. vignae*. There are, however, striking differences among chromosomes. The distribution of breaks in different chromosomes is illustrated in Fig. 10.

This diagram shows that *B. vignae* and *B. pseuderrans* are most similar in chromosomes 1, 2 and 5, whereas *B. vignae* and *B. eucaricis* are most similar in chromosome 4. The degree of similarity after reconstruction is also greatest when only a few obvious breaks have occurred. This seems clear for chromo-

Fig. 11. *Cerodontha* (*Butomomyza*) *angulata*. Chromosome maps. Description in the text. Brackets indicate the double inversions in chromosome 2 (arm 3 and 4).

somes 2 and 4. In chromosome 2, *B. pseuderrans* and *B. vignae* seem to be very similar as to banding pattern, whereas *B. vignae* and *B. eucaricis* display some regions with dissimilarities. In chromosome 4 the contrary is the case.

Cerodontha (*Butomomyza*) *angulata* (Loew)

The mitotic chromosome complement consists of two pairs of metacentric and one pair of submetacentric chromosomes. In polytene chromosome cells there are five long arms and one very small one. In contrast to the circumstances in the two *Trilobomyza*-species, which had also more or less metacentric chromosomes, it is easy to decide which arms belong to the same chromosome. In order to facilitate a discussion about homologies, the chromosome arms were also numbered separately in this species. The chromosome maps are also drawn with all centromeres to the left. Arms 1 and 2 form the largest chromosome, arms 3 and 4 the second one, and arms 5 and 6 the third one. The polytene chromosomes are illustrated in the chromosome maps, Fig. 11. This species seems to be variable in morphological traits (Nowakowski 1972) and the chromosomes also seem to be variable from population to population. These relationships are not quite clear. Thus, the chromosome maps presented here are representative only for a few populations. This is to some extent the case also for the following landmarks.

Arm 1 (chromosome 1 L). There is a broad, dark band, surrounded by more faintly stained bands (regions 12—13). There are also two puffs, situated in regions 18 and 22, which are as a rule recognizable.

Arm 2 (chromosome 1 S). There is a puff in regions 42—43, which is similar to region 42 in the species of the *pseuderrans* group. Also the double band in the border between regions 44 and 45 can be recognized here. The bands in regions 43 and 44 are,

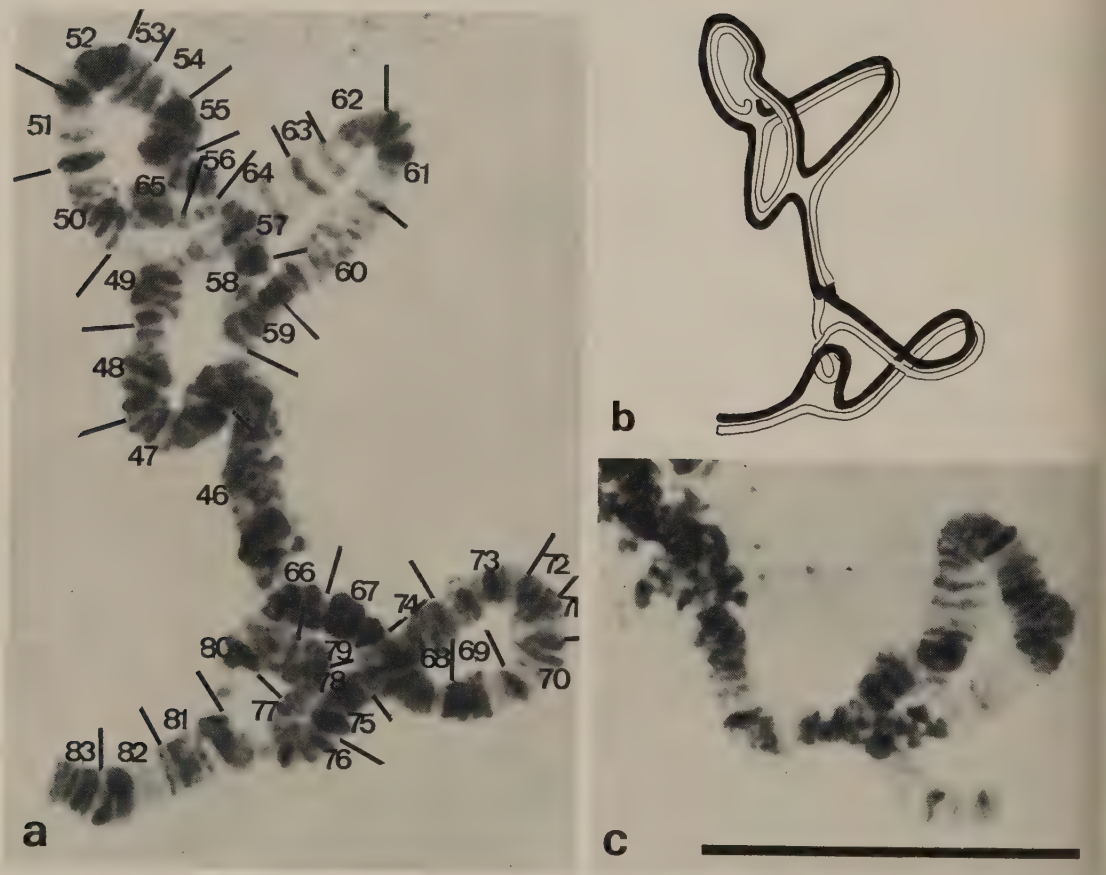


Fig. 12a—c. *Cerodontha* (*Butomomyza*) *angulata*. Heterozygous inversions. — a: Chromosome 2 heterozygous for double inversions in both arms. — b: Schematic interpretation of the complex figures. Black chromosome = standard gene arrangement, white chromosome = inverted gene arrangement. — c: Heterozygous inversion in the X chromosome (chromosome 3L, arm 5). This inversion is not indicated in the chromosome map, as both gene arrangements are different from that of the chromosome map. Scale 25 μ.

however, more deeply stained than the homologous ones in the *pseuderrans* group. A puff can often be seen in this region.

Arm 3 (chromosome 2 L). The same constriction as in the other *Butomomyza*-species is the best landmark. Here it is situated in the border between regions 56 and 57 in the chromosome map, but the position may be different due to inversions in this chromosome arm.

Arm 4 (chromosome 2 S). This chromosome is characterized most easily by the terminal segment, regions 82 and 83. These regions are similar to the terminal region of arm 4 in the members of the *pseuderrans* group. Regions 69 to 70 begin with a cluster of dark bands. There are two thin but deeply stained bands, one in the border between 69 and 70 and one near the end of region 70. The rest of this section contains only faintly stained bands.

Arm 5 (chromosome 3 L = X L). The terminal segment, regions 98—99, seems to be similar to the terminal segment in the X chromosome in the *pseuderrans* group. Regions 92 and 93 are also similar to regions in the *pseuderrans* group (region 95 in *B. vignae* and region 94 in *B. pseuderrans*).

Arm 6 (chromosome 3 S = X S). This small dot-like chromosome is smaller than in any of the other species. It contains the nucleolar organizing region but very few distinct bands.

In *Butomomyza angulata* inversion polymorphism has been found. In chromosome 2 L, a double inver-

ion of the overlapping type has been found. The two inversions involved have the four break points situated in regions 46, 49—50, 58 and 64—65. The double inversion thus covers nearly the whole chromosome arm. No intermediate gene arrangement has been found. In chromosome 2 S there is a double inversion, which might be either of the included or of the overlapping type. The break points are in regions 66, 77 and 80. Region 66 is near the centromeric heterochromatin and therefore it is not possible to decide whether there are two break points very close to each other or whether there is only one. If there are two break points, there might be a small overlapping region. The two double inversions in chromosome 2 are observed together in several individuals. They are illustrated in heterozygous condition in Fig. 12a, b. In the X chromosome one inversion has been observed in heterozygous condition. This inversion is observed in another population than those which are represented in the chromosome map. A comparison of the banding patterns indicates differences consisting of at least two inversions between the X chromosome map and both gene arrangements in the heterozygous inversion. Therefore, this inversion is not indicated by brackets in the chromosome map. The heterozygous inversion is illustrated in Fig. 12c.

Cerodontha (*Butomomyza*) *scirpi* (KARL)

In mitotic metaphase there is one very large telocentric chromosome pair, four smaller telocentrics and one dot-like pair.

In polytene chromosome cells the usual complement of five longer and one dot-like chromosome is visible. The chromosomes are illustrated in the chromosome maps, Fig. 13, and are characterized by the following landmarks.

Chromosome 1. Between region 7 and 8 there is a double band, which is easily recognized. In region 25 a puff is visible in fullgrown larvae.

Chromosome 2. There is a double band in the border between regions 40 and 41 and region 46 begins and ends with obvious dark bands.

Chromosome 3. The same constriction which occurs in other species of the genus is observed here in regions 56—57. The terminal segment, region 67, is also typical and shows similarities with the homologous region in the *pseuderrans* group.

Chromosome 4. Both the proximal and the terminal regions are characteristic for this chromosome. Region 68 begins with a constriction and a dark band, probably representing alfa-heterochromatin. Region 84 shows a puff and begins and ends with thin but distinct bands.

Chromosome 5. This is the X chromosome. It displays tendencies to the allocycly observed in some species of the family. There are large amounts of centromeric heterochromatin of the loose beta-type. The terminal region, 99, is similar to the same region in the *pseuderrans* group.

Chromosome 6. This small dot-like chromosome lacks remarkable bands.

All five members of the subgenus *Butomomyza* share some characteristic traits. The constriction in chromosome 3 is the most obvious landmark. In the X chromosome, which is relatively short, there are also some regions near the terminal end, which are common for all five species. In addition, *Butomomyza angulata* has some regions in chromosomes 2 and 4 similar to regions in the same chromosome in the *pseuderrans* group. There are also some regions in chromosome 3 in *Butomomyza scirpi* which are similar to regions in chromosome 3 in the *pseuderrans* group. There are, however, no common regions for *B. angulata* and *B. scirpi*, which do not occur in the whole subgenus. *B. scirpi* and *B. angulata* belong to different species groups.

Subgenus *Dizygomyza* HENDEL

Cerodontha (*Dizygomyza*) *ireos* (GOURREAU)

In mitotic cells there are six pairs of telocentric chromosomes.

The polytene chromosome complement consists of five long chromosomes and a small dot-like one. The polytene chromosomes are illustrated in the chromosome maps, Fig. 14.

Chromosome 1. The distal end, region 26, is characterized by three distinct bands, one at the beginning, one at the middle and one at the end of this region.

Chromosome 2. Both the proximal end, regions 27—28, and the distal end, regions 45—46 begin and end with dark bands. In the middle of all these regions there are only faintly stained bands.

Chromosome 3. This chromosome contains a constriction which is probably homologous to the constriction in the subgenus *Butomomyza*. Here it is situated in regions 56—57. This constriction is, however, less obvious in this species. The proximal end contains smaller regions with faintly stained bands and regions with a little more deeply stained but indistinct bands. These regions are limited by single distinct bands.

Chromosome 4. The distal end has a cluster of deeply stained bands in region 81, after that a light region, 82, which ends with two dark but diffuse bands.

Chromosome 5 (=X). Like many other X chromosomes this chromosome has only a few deeply stained bands.

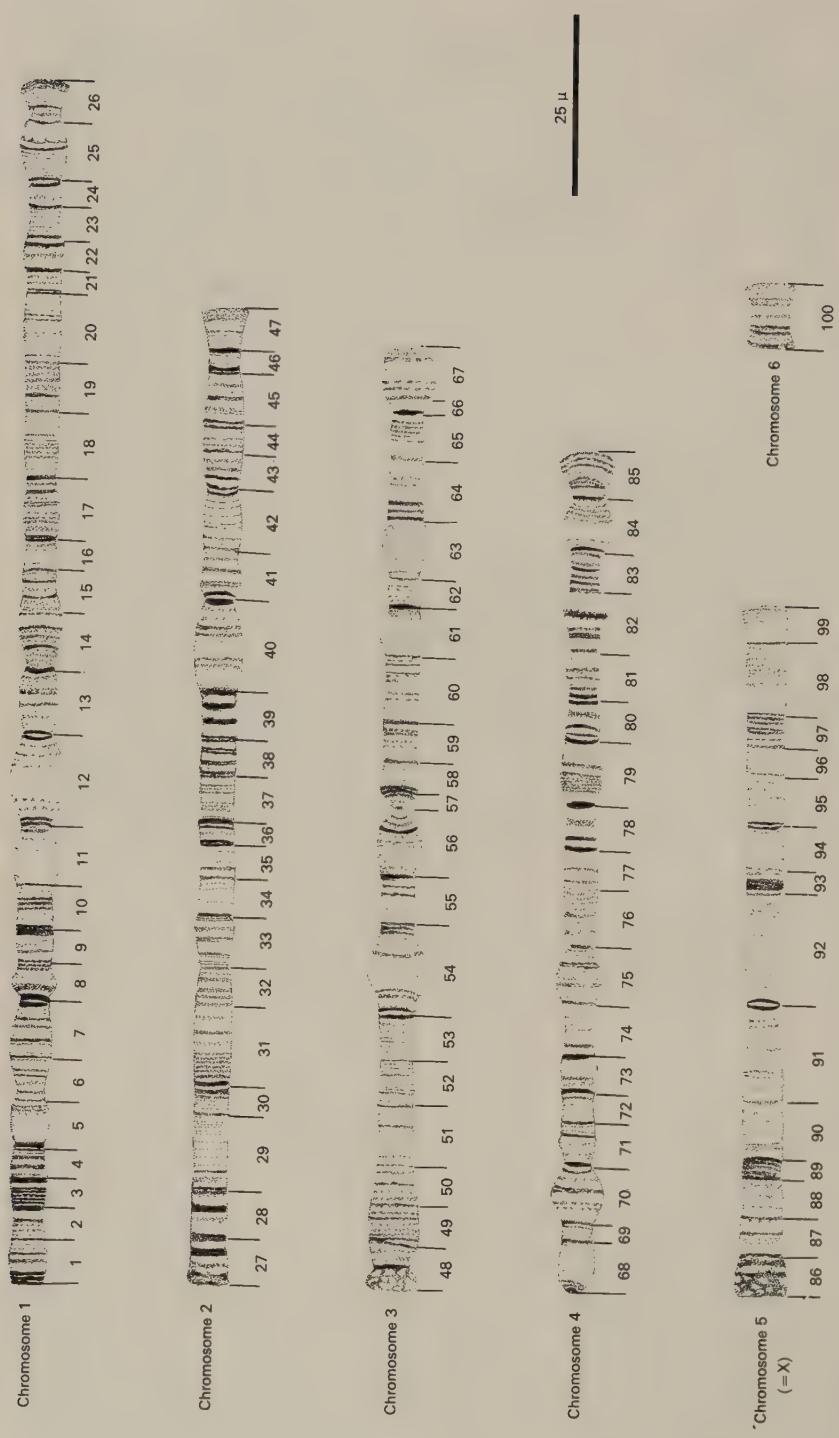


Fig. 13. *Cerodontha (Butomomyza) scirpi*. Chromosome maps. Description in the text.

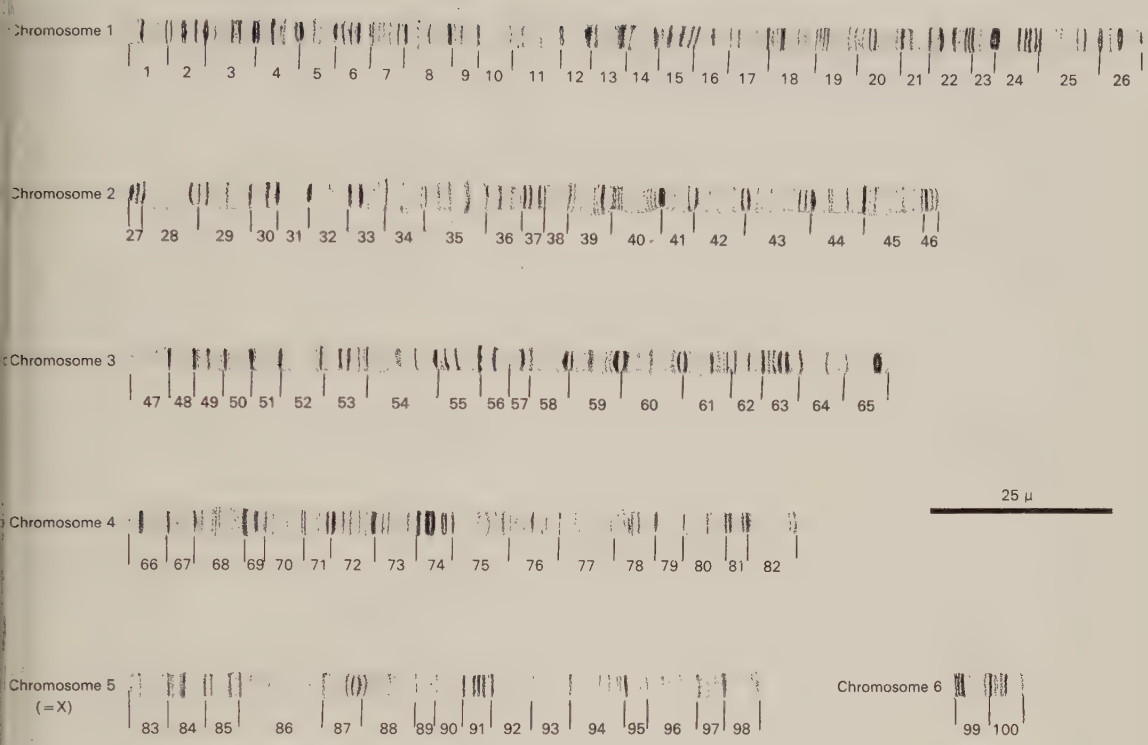


Fig. 14. *Cerodontha* (*Dizygomyza*) *ireos*. Chromosome maps. Description in the text.

Chromosome 6. This small dot-like chromosome contains a few distinct bands in both regions (99 and 100).

Cerodontha (*Dizygomyza*) *spinata* (GROSCHKE)

In mitotic cells there are six telocentric chromosomes.

In polytene chromosome cells there are five long arms and a small dot-like one. The polytene chromosomes are illustrated in the chromosome maps, Fig. 15.

Chromosome 1. There is a double band in regions 14—15 and a puff in regions 15—16.

Chromosome 2. The end segment, region 44, begins with one dark band and ends with four very distinct bands. There is also a double band in the border between regions 35—36.

Chromosome 3. In regions 52—53 there is a constriction similar to that of the other *Cerodontha*-species.

Chromosome 4. There are three dark bands in regions 70—71, two in regions 75—76 and one in regions 80—81.

Chromosome 5. This is the X chromosome, which contains fewer distinct bands than the large autosomes. The terminal segment, region 98, contains one thin but distinct band. The rest of the region contains only faintly stained bands.

Chromosome 6. This is the smallest chromosome, and, like the other dot-like chromosomes in the genus *Cerodontha*, it lacks distinct bands.

In this species there is a tendency to reduced pairing of homologous chromosomes. This reduced pairing is probably not due to structural heterozygosity as unpaired segments differ from cell to cell. One inversion has been found in the X chromosome. It has been observed in two females in heterozygous condition and in one male in hemizygous condition. The heterozygous inversion is illustrated in Fig. 16.

The two species belonging to the subgenus *Dizygomyza* display less similarity than the members of the subgenus *Butomomyza*. In chromosome 2, the terminal segment, region 44 in *Dizygomyza spinata* and regions 45—46 in *Dizygomyza ireos*, are similar. The constriction in chromosome 3, typical for both subgenera *Butomomyza* and *Dizygomyza*, is another similarity. No other similar segments are found.

A comparison of all members of the two subgenera

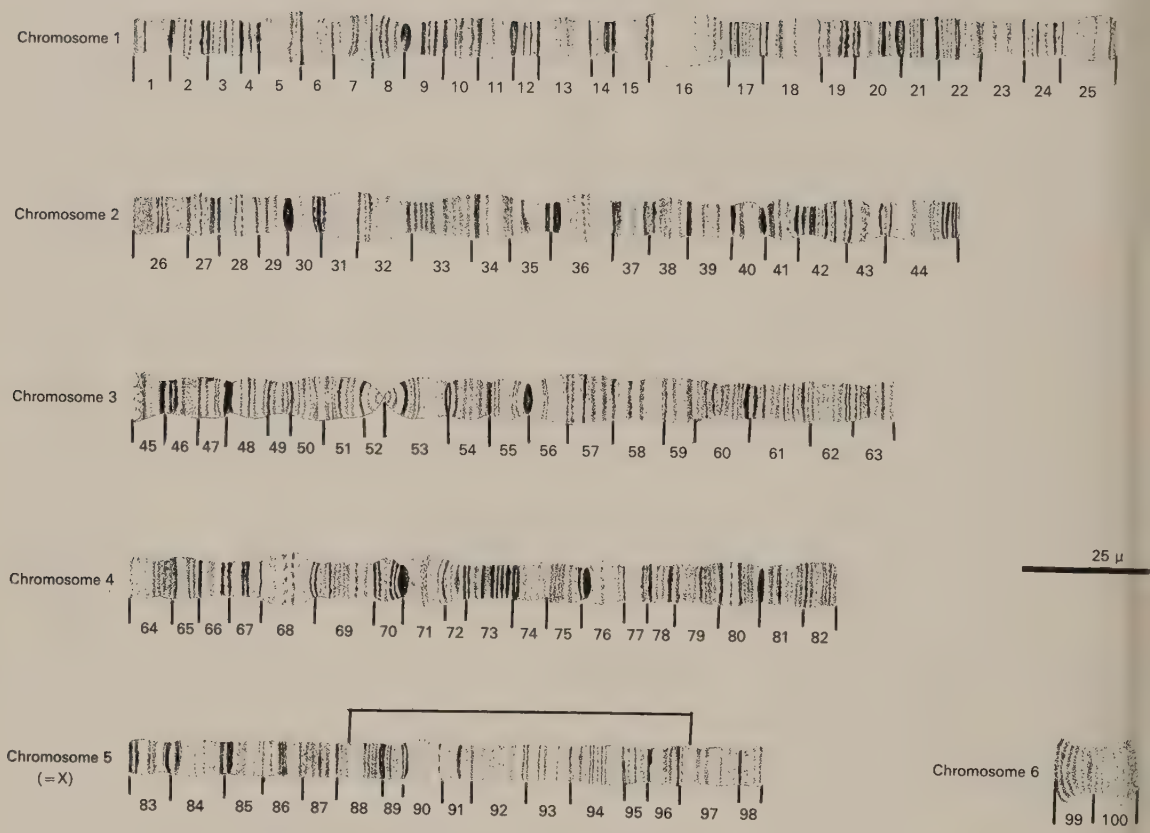


Fig. 15. *Cerodontha* (*Dizygomyza*) *spinata*. Chromosome maps. Description in the text.

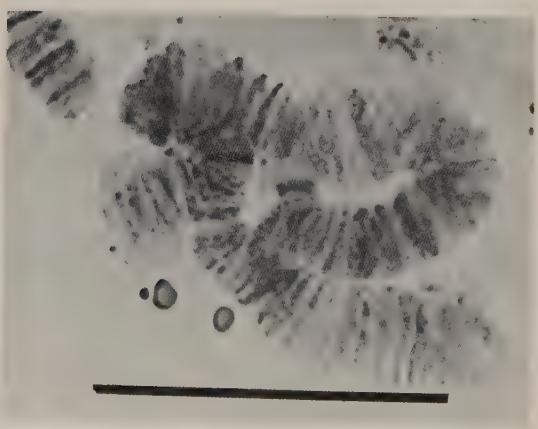


Fig. 16. *Cerodontha* (*Dizygomyza*) *spinata*. Heterozygous inversion in the X chromosome (chromosome 5). Scale: 25 µ.

shows that different species have some common traits as regards chromosome morphology. The relative lengths are similar, the X chromosome is no. 5 and the small dot-like chromosome is always present but has less distinct bands than in the genus *Phytomyza*. The only similar band sequences are, however, those connected with the constriction in chromosome 3.

2. Chromosomal differences and similarities in the whole family

A. *Mitotic chromosomes*

Table 2 shows the mitotic chromosome complements. Here *Liriomyza urophorina*, investigated by MAINX (1951), is included. No measurements were made on

Table 2. Mitotic chromosomes

Species	Somatic chromosome number	Haploid chromosome complement	Haploid number of major arms in mitotic cells
Phytomyza abdominalis			
Western semispecies	10	1 V and 4 R	6
Eastern semispecies	12	6 R	6
Phytomyza crassiseta	12, 18[1]	6 R	6
Phytomyza ilicis	12[2]	5 R and 1 D	5
Phytomyza primulae	12	5 R and 1 D	5
Amauromyza			
(*Trilobomyza*) *flavifrons*	6	2 V and 1 J	6
(*Trilobomyza*) *verbasci*	6	2 V and 1 J	6
Cerodontha			
(*Butomomyza*) *eucaricis*	12	1 V and 5 R	7
(*Butomomyza*) *pseuderrans*	12	5 R and 1 D	5
(*Butomomyza*) *vignae*	12	5 R and 1 D	5
(*Butomomyza*) *angulata*	6	2 V and 1 J	6
(*Butomomyza*) *scirpi*	12	1 large R, 4 small R, 1 D	5
(*Dizygomyza*) *ireos*	12	6 R	6
(*Dizygomyza*) *spinata*	12	6 R	6
Liriomyza urophorina[3]	12	5 R and 1 D	6

[1] Triploids
[2] In somatic cells; in gonial cells there are in addition a number of dot-like chromosomes
[3] According to MAINX (1951)
The designation for the chromosome complement are the usual for *Drosophila*
R = rod-shaped (telocentric) chromosome
V = V-shaped (metacentric) chromosome
J = J-shaped (submetacentric) chromosome
D = dot-like chromosome

mitotic chromosomes, as they are small and give comparatively little information. The most usual chromosome number is 2n = 12, which occurs in 11 species, including one of the two semispecies in *Phytomyza abdominalis*. Four of these species have five telocentric and one dot-like pair (five rods and one dot). This is in agreement with the usual polytene chromosome complement of five long arms and a very small one. Four species contain six telocentric pairs, which is one more major arm than in the polytene chromosome complement. This could indicate one heterochromatic arm. One species, namely *Butomomyza eucaricis*, has even seven major arms in mitotic cells. There is one very large metacentric chromosome and five smaller telocentric ones of about the same size. Here the amount of heterochromatin is still higher. The amount of heterochromatin has increased also in *Butomomyza scirpi*, where one of the telocentric chromosomes is much longer than all the other ones.

The chromosome number has decreased through one centric fusion to 2n = 10 in one semispecies in *Phytomyza abdominalis*, and to 2n = 6, through three centric fusions in *Trilobomyza flavifrons*, *T. verbasci* and *Butomomyza angulata*. In all these three species there are one submetacentric and two metacentric pairs. Thus, there are six major arms in mitotic cells compared with five in the polytene chromosome cells and therefore probably one arm is heterochromatic.

In the parthenogenetic species *Phytomyza crassiseta*, the chromosome number has increased by polyploidy to 2n = 18.

The differences and similarities in mitotic chromosome morphology do not seem to have any systematic importance. Members of the same genus may have different karyotypes, whereas similar karyotypes may occur in different genera. Thus, it is probable that an increase of constitutive heterochromatin and centric fusions have both occurred several times during evolution.

B. *Polytene chromosomes*

a. Length relationships. — Table 3 illustrates the relative lengths of the chromosome arms for the 13 species. Each chromosome arm is measured separately. Due to centric fusions the relative lengths of the chromosomes may be different. Different staining methods might cause differences in absolute lengths. This is the reason why absolute lengths are not stated in this table. The X chromosome may be either number 4 or 5. It seems to be adequate to compare all X chromosomes with each other and not all chromosomes 4 or all chromosomes 5. In *Phytomyza abdominalis*, western semispecies, and in *Butomomyza angulata*, the X chromosome has fused with the smallest autosome. In both cases X chromosome length in the table represents only the long arm. No measurements are presented for the smallest chromosome in *Trilobomyza flavifrons* and *T. verbasci*. It seems likely, however, that a dot-like chromosome might exist even in these species. In some cells of *Trilobomyza flavifrons* it is possible to trace a dot-like chromosome but never one with distinct bands. The difference between this type of morphology on the one hand and that of most *Cerodontha*-species on the other might be rather quantitative than qualitative.

Differences in relative lengths are tested statistically in Table 4. No intrageneric differences were found except for the smallest chromosome. This

Table 3. Relative lengths of polytene chromosomes in the 13 Agromyzidae species investigated

Species	Chromosome arm						
	1	2	3	X=4 or 5	4 or 5 (autosome)	6	No. of chromosomes
Phytomyza abdominalis	24.91	18.83	18.43	18.67	16.61	2.51	10
Phytomyza crassiseta	25.10	19.78	18.86	17.88	16.41	1.97	6
Phytomyza ilicis	25.91	19.49	19.17	17.50	16.23	1.71	6
Phytomyza primulae	24.50	19.61	19.34	17.57	17.19	1.80	7
Genus *Phytomyza* Mean ± SE	25.06 ± 0.31	19.35 ± 0.25	18.89 ± 0.17	18.05 ± 0.32	16.63 ± 0.18	2.06 ± 0.13	29
Amauromyza (*Trilobomyza*) *flavifr.*	25.48	21.95	20.16	15.57	16.83	—	10
Amauromyza (*Trilobomyza*) *verbasci*	25.60	20.44	20.39	15.44	18.02	—	6
Genus *Am.* (*Trilobomyza*) Mean ± SE	25.53 ± 0.33	21.38 ± 0.35	20.25 ± 0.31	15.52 ± 0.34	17.28 ± 0.34	—	16
Cerodontha (*Butomomyza*) *eucaricis*	25.79	20.59	19.67	14.08	17.40	2.48 ± 0.15	10
Cerodontha (*Butomomyza*) *pseuderr.*	25.19	19.82	19.81	15.19	18.01	2.03 ± 0.16	10
Cerodontha (*Butomomyza*) *vignae*	25.01	20.21	20.71	14.75	17.79	1.68 ± 0.13	10
Cerodontha (*Butomomyza*) *angulata*	25.13	20.44	19.77	15.79	17.86	1.17 ± 0.07	10
Cerodontha (*Butomomyza*) *scirpi*	25.64	20.68	19.98	14.52	17.78	1.39 ± 0.05	10
Genus *Cer.* (*Butomomyza*) Mean ± SE	25.35 ± 0.22	20.35 ± 0.18	19.99 ± 0.23	14.89 ± 0.23	17.77 ± 0.21	(1.75 ± 0.09)	50
Cerodontha (*Dizygomyza*) *ireos*	25.70	20.49	19.22	15.92	16.91	1.77	10
Cerodontha (*Dizygomyza*) *spinata*	24.53	20.59	19.12	16.11	17.78	1.87	10
Genus *Cer.* (*Dizygomyza*) Mean ± SE	25.12 ± 0.28	20.54 ± 0.32	19.17 ± 0.26	16.02 ± 0.28	17.35 ± 0.24	1.82 ± 0.05	20

chromosome was significantly different in different *Butomomyza*-species. The differences among genera are illustrated in Table 4B. Significant differences were observed for chromosome arms 2, 3, X and the smallest of the large autosomes. A pairwise comparison between genera (Table 4C) demonstrates a major difference between the genus *Phytomyza* and the other genera.

b. Band structure. — In the statistical comparison of relative chromosome length, chromosome structure is not considered. No identical band sequences of considerable length have occurred in species of different genera. Thus, it is not possible to know whether this comparison is really a comparison of homologous chromosomes. The X chromosome is probably homologous in different genera and might therefore be compared with more adequacy. Within genera, homologous segments are found within the genera *Amauromyza* and *Cerodontha* but not in *Phytomyza*. In those cases where it was possible to find homologous segments, it is clear that chromosomes of similar length also have a similar morphology. This is most apparent for the three *Butomomyza*-species belonging to the *pseuderrans* group. This observation makes the assumption probable that translocations have played a minor role in chromosomal evolution. It is not excluded, however, that some minor translocations, perhaps of the insertional type, might have occurred.

3. Intraspecific variation

There are three types of chromosomal variation within species: 1) polymorphisms, 2) incipient species differentiation (fixed inversions) and 3) parthenogenetic strains or clones with inversions fixed in heterozygous condition. Intraspecific variation is illustrated in Table 5.

For strictly cross-breeding organisms the number of heterozygous inversions per individual is the best measure for the degree of chromosomal polymorphism. Table 5 demonstrates that there are four species more polymorphic than the others. *Trilobomyza flavifrons* has 0.95 heterozygous inversions per individual, *Butomomyza angulata* has 0.85, *Butomomyza eucaricis* semispecies B has 0.71 and *Liriomyza urophorina* has 0.65. These four species (or semispecies) display as much polymorphism as polymorphic *Drosophila* species. They are, however, much less polymorphic than the most polymorphic populations in these *Drosophila* species.

The three species *Phytomyza primulae*, *Dizygomyza spinata* and *Butomomyza eucaricis* semispecies A. display much less polymorphism, but they are variable within populations. They display less than 0.1 heterozygous inversions per individual. Five species seem to lack chromosomal polymorphism, namely *Phytomyza abdominalis* (two semispecies), *Phytomyza ilicis*, *Trilobomyza verbasci*, *Butomomyza*

Table 4. Relative lengths of polytene chromosomes. Statistical test as to differences among and within genera

Chromosome arm	Source of variation	Sum of squares	df	Mean square	F
4A. Genus *Butomomyza*					
1	Among species	4.67	4	1.17	0.45
	Within species	117.00	45	2.60	
	Total	121.67	49		
2	Among species	4.81	4	1.20	0.70
	Within species	77.40	45	1.72	
	Total	82.21	49		
3	Among species	6.99	4	1.75	0.63
	Within species	124.35	45	2.76	
	Total	131.34	49		
X=4 or 5	Among species	16.16	4	4.04	1.77
	Within species	91.35	40	2.28	
	Total	107.51	44		
4 or 5 (autosome)	Among species	2.04	4	0.51	0.21
	Within species	108.85	45	2.42	
	Total	110.89	49		
6	Among species	11.10	4	2.77	18.51***
	Within species	6.75	45	0.15	
	Total	17.85	49		

Chromosome arm	Source of variation	Sum of squares	df	Mean square	F
4B. Total material					
1	Among genera	3.13	3	1.04	0.46
	Within genera	253.26	111	2.28	
	Total	256.39	114		
2	Among genera	45.79	3	15.26	8.37***
	Within genera	202.36	111	1.82	
	Total	248.15	114		
3	Among genera	32.58	3	10.86	5.90**
	Within genera	203.88	111	1.84	
	Total	236.46	114		
X=4 or 5	Among genera	167.68	3	55.89	25.08***
	Within genera	229.56	103	2.23	
	Total	397.24	106		
4 or 4 (autosome)	Among genera	23.90	3	7.97	4.74**
	Within genera	186.44	111	1.68	
	Total	210.34	114		
6	Among genera	1.95	2	0.97	0.54
	Among species	14.49	8	1.81	8.90
	Within species	17.92	88	0.20	
	Total	34.36	98		

Table 4C. Pairwise comparison between genera by *t*-analysis, using moan square within genera for s^2

Genera	Chromosome arm 1		2		3		X=4 or 5		4 or 5 (autosome)	
	t	df	*t*	df	*t*	df	*t*	df	*t*	df
Ph. — Tril.	0.995	43	4.835***	43	3.218**	43	5.327***	40	1.601	43
Ph. — But.	0.837	77	3.157**	77	3.477***	77	8.579***	69	3.762***	77
Ph. — Diz.	0.132	47	3.030**	47	0.716	47	4.579***	44	1.898	47
Tril. — But.	0.399	64	2.676**	64	0.663	64	1.445	59	1.322	64
Tril. — Diz.	0.809	34	1.864	34	2.367*	34	0.987	34	0.159	34
But. — Diz.	0.343	68	0.543	68	2.281*	68	2.797**	63	1.234	68

pseuderrans and *Butomomyza vignae*. For *Butomomyza scirpi* and *Dizygomyza ireos*, no conclusions can be drawn due to the low number of individuals investigated. Also for *Trilobomyza verbasci*, *Butomomyza pseuderrans* and *Butomomyza vignae* a very little pronounced polymorphism might exist.

Table 5 also demonstrates a striking difference between the two cases of incipient species differentiation. In *Phytomyza abdominalis* no structural heterozygosity occurs except in hybrids and hybrid descendants. In hybrids there are as much as 4.7 heterozygous inversions per individual. In *Butomomyza eucaricis*, on the contrary, both semispecies are polymorphic, although one of them considerably more than the other. Only one fixed inversion distinguishes the two semispecies, but all polymorphisms are different. Thus, hybrids do not contain much more heterozygosity than the most polymorphic semispecies.

Phytomyza crassiseta contains both the highest number of inversions and the highest number of heterozygous inversions per individual. This is due to the fact that this species is parthenogenetic and has thus preserved much heterozygosity. Especially the triploids have great possibilities to obtain heterozygosity. In a parthenogenetic species the number of heterozygous inversions per individual is not a good measure of the degree of polymorphism. In spite

Table 5. Intraspecific variation

Species	No. of individuals investigated	No. of polymorphic inversions	No. of other inversions	Heterozygous inversions per individual	Type of variation other than polymorphism
Phytomyza abdominalis[1,4,6]					
Western semispecies	1,838	0	0	0	
Eastern semispecies	773	0	0	0	
Hybrids (F_1 and back-cr.)	49	0	7	4.73	Incipient species form.
Total	2,660	0	7	0.09	Incipient species form.
Phytomyza crassiseta[2,6]					
Triploids	462	0	9	2—4	Parthenogenetic strains
Diploids	15	0	2	0—1	Parthenogenetic strains
Phytomyza ilicis[6]	400	0	0	0	
Phytomyza primulae[6]	138	1	0	0.01	
Amauromyza					
(*Trilobomyza*) *flavifrons*[5]	762	8	0	0.95	
(*Trilobomyza*) *verbasci*[6]	92	0	0	0	
Cerodontha					
(*Butomomyza*) *eucaricis*[3,6]					
Semispecies A	1,382	2	0	0.06	
Semispecies B	472	3	0	0.71	
Hybrids (F_1 and back-cr.)	49	5	1	1.47	Incipient species form.
Total	1,903	5	1	0.25	
(*Butomomyza*) *pseuderrans*[6]	111	0	0	0	
(*Butomomyza*) *vignae*[6]	91	0	0	0	
(*Butomomyza*) *angulata*[6]	20	5	0	0.85	
(*Butomomyza*) *scirpi*[6]	10	0	0	0	
(*Dizygomyza*) *ireos*[6]	32	0	0	0	
(*Dizygomyza*) *spinata*[6]	26	0	0	0	
Liriomyza urophorina[7]	1,833	6	0	0.65	
Grand total	8,655	26	19		

[1] BLOCK 1969a [2] BLOCK 1969b [3] BLOCK 1974 [4] BLOCK 1975a [5] BLOCK 1975b [6] This paper
[7] MAINX et al. 1956

of the high number of heterozygous inversions per individual there are only four different types in the triploids and two in the diploids.

There could be some reason for splitting this "species" into two or three, depending on polyploidy and the kind of reproduction. This has not been done.

In all three kinds of intraspecific variation, paracentric inversions are the predominating structural changes. Apart from inversions, small changes in single bands have been found in *Phytomyza abdominalis*, *Phytomyza crassiseta* and *Trilobomyza flavifrons*. Thus, there is no difference among the three types of variation as to types of structural rearrangements. Complex inversions are found as polymorphisms in *Trilobomyza flavifrons* and as fixed inversions differentiating the two semispecies in *Phytomyza abdominalis*. Complex inversions are also found in *Butomomyza angulata*, but the kind of variation is a little doubtful.

Discussion

1. Relationships among species

A. *Mitotic chromosomes*

STONE (1962) gives a survey of the changes in chromosome structure which have reached fixation in *Drosophila*. Among these, the following were possible to detect in mitotic chromosomes: 58 centric fusions, 38 additions of heterochromatin, 32 pericentric inversions and 3 translocations. This consideration is founded on 300 species, which were investigated at the time. In Agromyzidae, only 14 species are investigated and thus, the material is perhaps not representative. It indicates, however, 6 centric fusions, 8 additions of heterochromatin and probably no translocations. The role of pericentric inversions is more doubtful. There are certainly no pericentric inversions large enough to be detected in mitotic chromosomes. But in two cases, namely in *Phytomyza ilicis* and *Phytomyza primulae*, there are indications in the

polytene chromosomes, that pericentric inversions might have become fixed. In *Phytomyza ilicis* chromosome 2 contains a heterochromatic segment in the border between regions 28 and 29. This segment is often attached to the loose common chromocentre. It might represent centromeric heterochromatin. If this be the case, the centromere might have changed its position from the usual terminal position through a pericentric inversion. In *Phytomyza primulae*, the small dot-like chromosome contains centromeric heterochromatin in the border between regions 99 and 100. Thus, this chromosome seems to be metacentric in contrast to all other dot-like chromosomes observed.

Centric fusions seem to have played the same role here as in *Drosophila* and the same chromosome number is dominant. There are three independent cases: in *Phytomyza abdominalis*, the X chromosome and the dot-like one have fused in the western semispecies, in the two *Trilobomyza*-species, arms 1 and 3 and arms 2 and 4 have fused. The occurrence of the same fusions in two closely related species probably means that this change has occurred only once. *Butomomyza angulata* has also three pairs of chromosomes and a similar mitotic complement. But the metacentric chromosomes contain another composition of arms. Thus, these centric fusions must be independent. In this manner, altogether 6 centric fusions have occurred.

Additions of heterochromatin, which are common in *Drosophila*, seem to be still more usual in Agromyzidae. In *Butomomyza eucaricis* there are seven major arms. These seven arms occur as one very large metacentric chromosome and five much smaller telocentric ones. This implies that most of the large pair in mitotic metaphase is heterochromatic. In *Phytomyza abdominalis*, one arm and a large segment of the other one in the same chromosome is heterochromatic, (Block 1975a). In *Butomomyza scirpi*, there are five major arms, but one of them is extremely large. In the six remaining cases there are six pairs of telocentric chromosomes instead of five rod-like and one dot-like pair — a usual karyotype in *Drosophila* too. In the two *Trilobomyza* species the extra heterochromatin might have a common origin. The extra chromosomes in the germ-line of *Phytomyza ilicis* have no correspondence in the genus *Drosophila*. Such chromosomes are, however, observed in other Diptera. If these chromosomes are heterochromatic or not, is impossible to decide, as they do not occur in the polytene chromosome cells.

The absence of translocations is also in agreement with the circumstances in *Drosophila*.

B. *Polytene chromosomes*

In the genus *Drosophila* many species are investigated as to polytene chromosomes. A detailed homologization, however, is possible only in those species groups which have changed relatively little during evolution. Examples of such groups are the *virilis* group (Stone et al. 1960), the *repleta* group (Wasserman 1963), the *melanica* group (Stalker 1966), the Hawaiian species groups (Carson and Stalker 1968a, b, c, 1969) and the *robusta* species group (Narayanan 1973). In those species groups where more comprehensive structural rearrangements have occurred, the relationships are more difficult to reconstruct. Homologous segments in different species groups are still more difficult to identify. Stalker (1972) compared a number of species belonging to different species groups. Homologous segments could be detected in the most "primitive" species in different species groups. Thus, he was able to reconstruct the most likely phylogenetic relationships among these groups.

Also in Simuliidae it has been possible to decide which structural rearrangements distinguish nearly related species. These investigations concern the genus *Prosimulium* (Basrur 1959, 1962; Ottonen 1966) and *Eusimulium* (Dunbar 1959). In the genus *Chironomus* (family Chironomidae), Keyl (1962) has managed to reconstruct a phylogenetic diagram including 21 species. Paracentric inversions have dominated in all these cases. All investigations concern groups of species where the chromosomes have changed relatively little during evolution. Even within species groups in the genus *Drosophila* it is not always easy to reconstruct evolutionary relationships. In those groups where many inversions distinguish closely related species, it is impossible to tell exactly which rearrangements underlie species differentiation.

In the present material the 13 species belong to three different genera. Even those from the same genus are not always closely related. This is especially the case for the genus *Phytomyza*, which is a very large genus, and where only a few species have chromosomes favourable enough for a detailed analysis. In those cases where no homologies can be traced, measurements are the only means of comparing chromosomes from different species. These measurements support the observation that translocations have played a minor role — if any at all — in chromosomal evolution. The same conclusion can be drawn from a comparison of the chromosomes in species from different genera. There are significant differences in relative lengths, but these

differences are small. This is also the case for *Liriomyza urophorina*, investigated by MAINX (1951). His paper does not include any measurements of the chromosomal lengths and therefore this species cannot be involved in the statistical treatment of length relations. Chromosomes 1 and 6 have about the same relative length as chromosomes 1 and 6 in the other genera. The length of the other four chromosomes is difficult to judge, as it is not clear which of them represents the X chromosome. Anyhow, differences must be small.

The comparison of three *Butomomyza*-species and of two *Trilobomyza*-species gives still more support to the assumption that intrabrachial rearrangements have been dominant. There are, however, important limitations in this type of comparison. First, there is a limitation in size for segments, which are possible to compare in this way. Secondly, small changes in single bands of the type that KEYL (1964, 1965, 1966) has described as differences between two subspecies in *Chironomus thummi* as well as differences in the puffing pattern could confuse the comparison. Thirdly, LEVENE and VAN VALEN (1962) have observed a single recessive gene, that changes the banding pattern in a small segment of the third chromosome of *Drosophila pseudoobscura*. Such changes might have happened several times and obscured the real structural rearrangements. This is the reason why no detailed analysis has been made concerning the inversions and shifts that distinguish the species, but only a rough estimation of the number of breaks that might lie behind chromosomal differences. This number could be too high or too low.

Comparisons of closely related species show that intrachromosomal changes have been dominating in both *Butomomyza* and *Trilobomyza*, and that these changes have been considerable. In both genera chromosomal differences are nearly as frequent as between *Drosophila pseudoobscura* and *D. miranda* (DOBZHANSKY and TAN 1936). Apart from paracentric inversions, which are probably the dominating type of structural rearrangement, homobrachial shifts, small duplications and deletions are probably involved. Changes in single bands, which might be regarded as a type of duplications or deletions, have probably been numerous. These types of changes are also found within species (BLOCK 1969b, 1975b). In addition, repeats, each involving a few bands, could have played some part. Indications that such changes have occurred are found in the three *Butomomyza*-species. Thus, region 47 in chromosome 3 and region 65 in chromosome 4 contain more bands in *Butomomyza vignae* than the homologous regions in the other two species in the same species group. The two *Trilobomyza*-species, which have been compared in the same way, displayed still more differences in the banding pattern. Probably, there are many differences in single bands, which can cause some difficulties in homologizing the chromosomes.

2. Intraspecific variation

A. *Polymorphisms*

As a general rule, species with a high degree of ecological adaptability are polymorphic, whereas species with ecological restriction are monomorphic (DOBZHANSKY et al. 1950; DA CUNHA et al. 1953). Although not without exceptions, this rule is still valid. To some extent it has some relevance also for Agromyzidae. The most polymorphic species investigated as to chromosomal polymorphism is *Trilobomyza flavifrons*. This is true both as regards the number of polymorphic inversions and the number of heterozygous inversions per individual. This species has a wider range of host plants, having been collected from many different genera within Caryophyllaceae. It has also been recorded from Chenopodiaceae, but no such material has been cytologically investigated. *Liriomyza urophorina* (MAINX 1951; MAINX et al. 1956) displays polymorphism in spite of being restricted to one plant species, *Lilium martagon*. If other ecological factors are considered, however, this species could be regarded as ecologically adaptable. There was, however, no correlation between environmental factors and chromosomal polymorphism. In *Drosophila flavopilosa*, a fly restricted to *Cestrum parqui*, BRNCIC (1962, 1967, 1968) has shown that polymorphism has an obvious correlation to environmental factors. The two species *Butomomyza eucaricis* and *B. pseuderrans* often occur in the same locality and their ecology seems to be similar. Nevertheless, *B. eucaricis* is polymorphic and *B. pseuderrans* is not. According to NOWAKOWSKI (1973), however, *B. pseuderrans* is much more rare and also restricted to a single plant species, *Carex hirta*, whereas *B. eucaricis* could be found in several species of *Carex*. More surprising is the difference in degree of polymorphism between the two semispecies within *B. eucaricis*. These two semispecies are found at the same localities and even in the same leaf (BLOCK 1974). Both are polymorphic, but one of them remarkably more than the other. The frequencies vary as to locality and in most places the less polymorphic semispecies dominates. *Butomomyza angulata*, the fourth species with a considerable degree of chromosomal polymorphism

is also a common species. According to NOWAKOWSKI (1973), it has a high degree of ecological adaptability and can be found in many species of *Carex* and also in *Scirpus silvaticus*. The present material has been collected only from *Carex hirta*. NOWAKOWSKI (1972) has observed morphological variation as to populations in this species. The polytene chromosomes also show a tendency towards this type of variation. Perhaps it is a polytypic species. This is not possible to decide from the small material available as yet. *Phytomyza abdominalis* and *Phytomyza ilicis* are both host species specific and both lack chromosomal polymorphism. A great number of individuals have been investigated from both species. The material of *Phytomyza abdominalis* has been collected from a great number of localities and different types of biotopes. *Phytomyza ilicis*, on the other hand, has been collected only from a few localities and only in gardens. Thus, it is not possible to characterize this species as monomorphic with the same certitude as *Ph. abdominalis*. No polymorphism has been found in *Trilobomyza verbasci*. This species should, according to Hering's terminology be characterized as oligophagous, as it has been observed in two genera, *Verbascum* and *Scrophularia*. It is remarkable that it occurs only in two, not very closely related genera of the family Scrophulariaceae. Thus, it might be possible, that *T. verbasci* in reality comprises two sibling species, each restricted to one plant genus. In such a case, monomorphism is also more plausible.

Thus, it seems, that the general rule of a connection between chromosomal polymorphism and ecological adaptability could be valid in most cases for Agromyzidae too. The exceptions from this rule, however, need more investigation.

B. *Fixed inversions due to incipient species differentiation*

There are two cases of incipient species differentiation in this material, *Phytomyza abdominalis* and *Butomomyza eucaricis*. In both cases — which are described in separate papers — differentiation has proceeded so far that it is a matter of definition whether the two "semispecies" within each species should be regarded as separate species. There are some similarities between these cases and the Hawaiian species *Drosophila setosimentum* and *D. ochrobasis* (CARSON et al. 1975). In all three cases, gene exchange occurs in natural populations. There are, however, important differences among the three cases. In both *Drosophila* and *Butomomyza* there are two polymorphic semispecies or sibling species. In *Phytomyza*, no chromosomal polymorphism can be found in any of the two semispecies. On the other hand, there are several fixed inversions. This difference is illustrated in Table 5. In *Butomomyza*, hybrids and hybrid descendants are not much more heterozygous than individuals belonging to the most polymorphic semispecies. In *Phytomyza*, hybrids or hybrid descendants are heterozygous for as much as 5 inversions in contrast to the "pure" semispecies, which are not heterozygous for any inversions at all. According to BOCK (1971), chromosomal differences among species are more complex in species groups of *Drosophila*, where chromosomal polymorphism is common. This is not the case for the two species pairs or species complexes in Agromyzidae.

Another difference is found in the distribution pattern. In *Phytomyza abdominalis* the two semispecies have a different distribution. In the natural border zone they can occur sympatrically, but hybridization is very rare in these localities. In one locality, one of the two semispecies has been introduced to the area of the other one. This has probably happened fairly recently. In these localities, hybridization is much more common. In the other two cases, no real differences in distribution of the two species or semispecies could be observed. Thus, gene exchange might be expected as a normal event, occurring regularly. The frequency of this gene exchange is, however, not so high as to cause a fusion of the gene pools.

C. *Inversions fixed in heterozygous condition in connection with parthenogenesis*

One single species has been cytologically investigated, namely *Phytomyza crassiseta*. A high number of heterozygous inversions per individual are observed in triploid individuals of this species. This probably depends on a preservation of inversions which had a selective advantage before parthenogenetic reproduction evolved. Inversions might have maintained the heterotic effect, and thus, strains, heterozygous for inversions might have been selected for. This seems to be the most likely explanation. If the structural heterozygosity had evolved after the origin of parthenogenesis, other types of structural changes would probably have been found, above all reciprocal translocations. PORTER (1971) has discussed the origin of polyploidy in connection with the parthenogenetic chironomid *Lundstroemia parthenogenetica*. He presumes that in a case like *Phytomyza crassiseta* triploidy might have arisen through a hybridization of diploid parthenogenetic and diploid sexual strains. The small material from diploids in the present material does not support this assump-

tion. The inversions found in the diploids are not identical with any of those found in the triploids. This is not strong evidence, however, as the diploid material is very small and it is not certain whether these individuals are parthenogenetic.

3. The connection between intra- and interspecific variation

Chromosomal polymorphism is not uncommon in Agromyzidae. In the cytologically investigated species, 25 polymorphic inversions have been observed. In 13 species — the parthenogenetic *Phytomyza crassiseta* not included — this makes 1.9 polymorphic inversions per species. This can be compared with 1.1 inversions per species in the *repleta* group in the genus *Drosophila*, (WASSERMAN 1963). The repleta group is one of the least polymorphic groups in the genus *Drosophila*. Thus, the family Agromyzidae is not very much polymorphic compared with the entire genus *Drosophila*. Nevertheless, a high number of inversions have probably become fixed during evolution, since even closely related species show great dissimilarities as to banding pattern. BOCK (1971) has assumed that groups of species with many differences in banding pattern among species are also very polymorphic. This assumption does not seem to be valid here. There are at least two possible explanations for this dissimilarity. First, most of the material was collected from Scandinavian localities, far from the distribution centre of the species, and marginal populations are as a rule less polymorphic than central ones. The second explanation is that there has been more chromosomal polymorphism at an earlier stage of evolution. This polymorphism might have reached fixation through a splitting of the earlier species. This splitting might be combined with a higher host plant specificity, which should lead to a weaker selection for new polymorphisms.

4. Sex determination

Mitotic chromosome numbers are always the same in both sexes. Thus, sex determination must be of the XY and not of the XO-type. MAINX (1951) and MAINX et al. (1956) did not find any chromosomal differences between males and females of *Liriomyza urophorina*. From the population data, the authors have come to the conclusion that there is a chromosome with the genetical function of a Y chromosome. None of the inversions is situated in this chromosome. This implies that the X chromosome ought to be one which does not contain significantly more than 25% heterozygotes for a certain inversion. Therefore, the X chromosome ought to be either number 4 or number 5. In the present material it has been established for a number of species that the Y chromosome is heterochromatic. Even in the small mitotic chromosomes it is often possible to see that one chromosome in a pair is shorter and more intensely stained. In the polytene chromosomes the morphology may be different in different species, and there is also variation among individuals in the same species. Often the two X chromosomes in the female are not broader than the single X of the male. This might be the case in *Liriomyza urophorina*. In those species where the X chromosome might display polymorphism, it nevertheless seems obvious that the Y chromosome is heterochromatic. In *Phytomyza abdominalis*, F_1 hybrids of the two semispecies display reduced pairing, if they are females but not if they are males (BLOCK 1969a). In *Trilobomyza flavifrons* a change in a single band may be seen in heterozygous condition in the females (BLOCK 1975b). In the males the deeply stained band is either absent or the whole chromosome has it. This might indicate that the X chromosome is single in the male sex. The same type of indication is obtained from *Dizygomyza spinata*. Here, an inversion is observed in two females in heterozygous condition and in one male in hemizygous condition. This indicates a heterochromatic Y.

Thus, sex determination is in principle of the same type as in *Drosophila*. There is, however, a morphological difference: in males of certain species of Agromyzidae the single X chromosome is often shorter and contains less distinct bands than the double X of the females. This difference between male and female X chromosomes is illustrated in Fig. 17 for the four species *Phytomyza abdominalis*, *Phytomyza ilicis*, *Phytomyza primulae* and *Butomomyza eucaricis*. WOLF (1957, 1968) observed a similar morphology in the X chromosome of *Phryne cincta*. In this case, however, even the double X of the female could display this changed morphology, which is an expression of allocycly. In *Phryne* the morphology of the X chromosome was dependent on temperature: at a low temperature, the X chromosome was long and had distinct bands; at a higher temperature, it was short and had indistinct bands. The single X chromosome of the male was always short and had indistinct bands. In Agromyzidae, no investigation has been carried out concerning the role of temperature or other environmental factors. They might influence the morphology of the male X chromosome since there are variations from individual to individual in the degree of contraction.

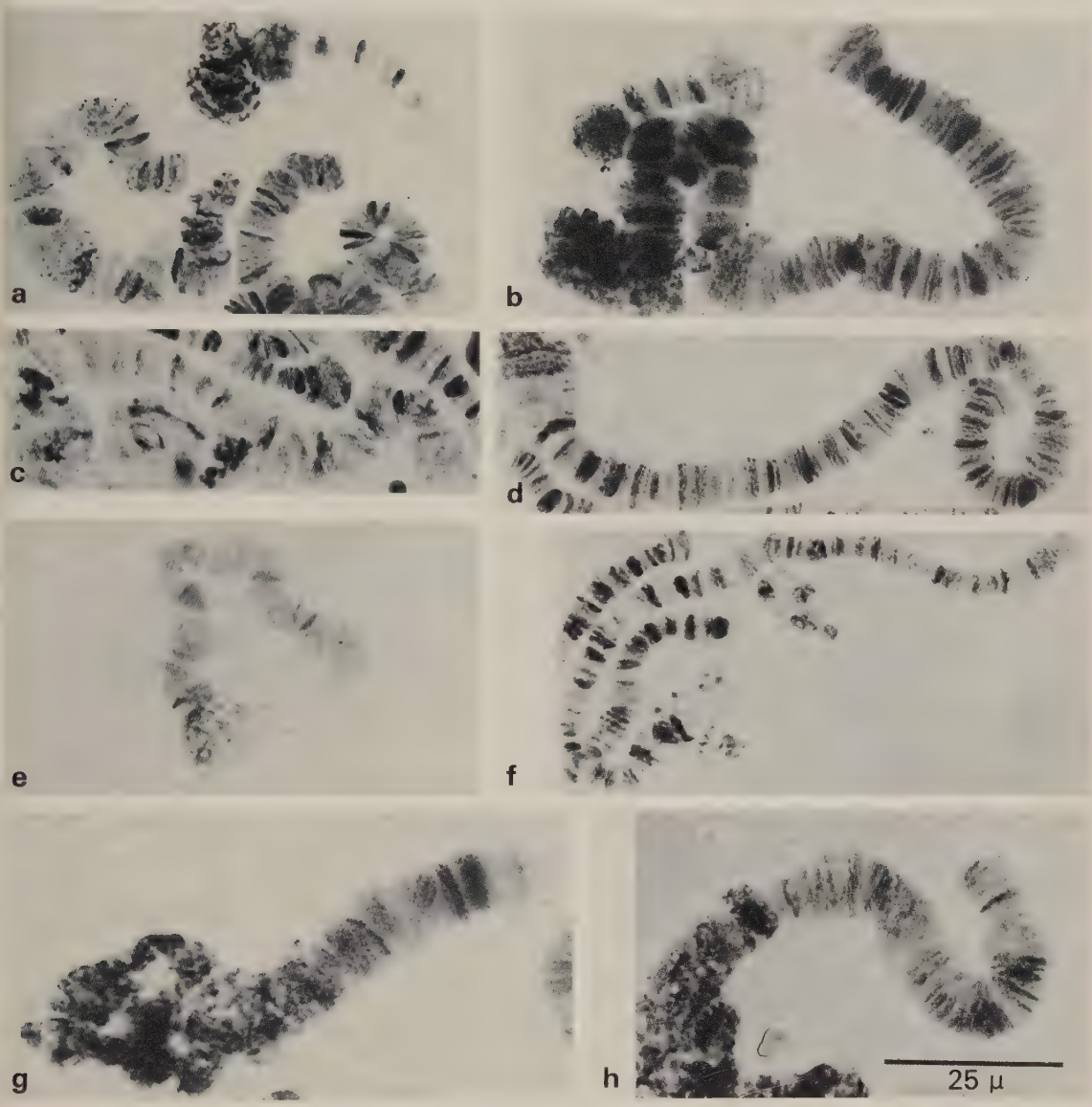


Fig. 17a—h. The X chromosomes in males and females of different species; a: *Phytomyza abdominalis*, male single X chromosome; b: the same species but female double X chromosome; c and d: *Phytomyza ilicis*; c: male; d: female X chromosome; e and f: *Phytomyza primulae*; e: male; f: female X chromosome; g and h: *Cerodontha* (*Butomomyza*) *eucaricis*; g: male; h:female X chromosome. Scale: 25 μ.

Females of Agromyzidae, however, always seem to have distinct bands.

In *Drosophila*, dosage compensation for the male X chromosome has been studied by KORGE (1970), by MARONI and PLAUT (1973) and by LAHKOTIA and MUKHERJEE (1969). The single X of the male seemed to have the same metabolic activity as the two X chromosomes of the female. In *Drosophila*, however, there is usually no difference as to distinctness of the bands between male and female X chromosomes. The single X is as a rule much thinner and only a little shorter.

The changed morphology of the single X in the male could be an indication of dosage compensation.

Short chromosomes with a diffuse banding pattern could represent a stage between polyploidy and polyteny. Such cells were observed by MATUSZEWSKI (1965) in the salivary glands of *Dasyneura urticae* and a few other gall midges. Similar cells are also observed in infected cells of *Rhynchosciara angelae* and other sciarids (DA CUNHA et al. 1968; PAVAN and DA CUNHA 1969; PAVAN et al. 1969). In these infected cells there is also a tendency to dissociation of the chromosomes, which always starts with the X chromosome (DA CUNHA et al. 1968). Similar changes occur in *Butomomyza eucaricis* in fat bodies but without traces of infection (BLOCK, unpubl.). Here, a similar dissociation always begins with the X chromosome in male cells. In female cells the smallest autosome is first dissociated and the X chromosome follows as number 2. In both male and female cells, the large autosomes are most rarely dissociated. This dissociation has been interpreted as an indication of high metabolic activity, which is an advantage in the infected cells. It is thus not unlikely that the structure with indistinct bands in the males could be an indication of high metabolic activity. This type of structure might be the cytological evidence of dosage compensation. This cannot be verified, however, without autoradiographical studies.

5. Chromosomes and taxonomy

The family Agromyzidae is divided into two subfamilies: Agromyzinae and Phytomyzinae. All species in the present investigation belong to Phytomyzinae. Three different genera are represented. Length relationships of the polytene chromosomes indicate that the genera are natural entities also from the cytological point of view. The genus *Phytomyza* is the most dissimilar as to relative lengths of the polytene chromosomes. This is also in agreement with taxonomical experience. The other genera have been regarded by earlier taxonomists as subgenera within a large genus. This genus was called *Dizygomyza* by HENDEL (1931) and *Phytobia* by FRICK (1952). NOWAKOWSKI (1962) has split this genus into several genera. Although length relationships might support the earlier opinion, this is not a strong indication. A comparison of the two subgenera *Dizygomyza* and *Butomomyza* and the genus *Amauromyza* indicates that *Butomomyza* is most different as to chromosomal lengths. This is not in agreement with morphological observations. *Butomomyza* and *Dizygomyza* are closely related subgenerna within the genus *Cerodontha*. According to SPENCER (1971), it is not even justified to regard them as different subgenera. NOWAKOWSKI (1973) regards them as different subgenera, although very closely related. Length relationships give only a rough estimation of the real relationships and can at most support NOWAKOWSKI's opinion of two different subgenera. The two subgenera have at least one region with similar band sequences, which could not be found in the genus *Phytomyza*.

The most useful taxonomical information is obtained from cytological studies of very closely related species and from studies on intraspecific variation. The two cases of incipient species differentiation indicate that very detailed information can be obtained from studies of polytene chromosomes. In neither of these cases was there any reason to split the species, if only morphological characters were regarded. In one of the cases (*Phytomyza abdominalis*) morphological differences have been detected later, which might support the cytological observations. Thus, it is probable that cytogenetic studies could be a useful complement to morphological methods in solving taxonomical problems in the family Agromyzidae. Due to the inferior quality of the polytene chromosomes in many species, it is not likely, however, that a complete cytological description of all species morphologically described will ever be performed.

General conclusions

Chromosomal evolution in Agromyzidae can be studied in both mitotic chromosomes and in polytene chromosomes. Mitotic chromosomes give more information about changes in the amount of heterochromatin and about centric fusions (or fissions). Both types of changes seem to have played a great role in species differentiation. It seems most likely (although not certain) that the most "primitive" karyotype is "five rods and one dot" as is the case in the genus *Drosophila* (PATTERSON and STONE 1952). This means that the amount of heterochromatin has probably increased in a number of species, and that centric fusions and not fissions have taken place.

The polytene chromosomes give much more information on chromosomal evolution. It is not possible to characterize certain species as "primitive". The structural rearrangements, which can be traced in a comparison of closely related species are: paracentric inversions, homobrachial shifts, changes in single bands and perhaps also repeats. Pericentric inversions have probably occurred, but they have played a minor role. There are several

reasons to presume that reciprocal translocations have had very small importance, if any at all.

In the mitotic chromosome complement there is no case where one of the rod-like chromosomes is longer and at the same time one is shorter than the remaining ones. All differences in length can be explained by changes in the amount of constitutive heterochromatin. This is not strong evidence, however, as mitotic chromosomes are small and no measurements of them have as yet been made.

The comparison of relative lengths of the polytene chromosomes indicates a great constancy as to length relations. Even species belonging to different genera have similar relative lengths in their polytene chromosomes. The small differences that exist can be explained by minor duplications and deletions.

The comparison of closely related species makes it possible to reconstruct most of the structural rearrangements, which have occurred in species differentiation. There are, however, some segments that have apparently no similarities in closely related species. In no case, are such segments similar to segments in other chromosomes in the other species.

Reciprocal translocations are the type of structural rearrangements that have the most severe effects on fertility of the heterozygotes. Double paracentric inversions as well as tandem shifts have a smaller effect. These types of rearrangement are also found floating within populations. Thus, their positive effects might be large enough to compensate for the negative effect on fertility.

Chromosomal rearrangements in the present material are, therefore, of the same type whether they occur as intraspecific or as interspecific differences. This means that chromosomal differences play a minor role, if indeed any, in reproductive isolation between species. Due to the detailed structure of the polytene chromosomes, chromosomal studies are, however, of great value for both taxonomical and genetic investigations.

Acknowledgments. — My sincere thanks are due to Professor Arne Lundquist for valuable discussions and for critical reading of the manuscript, to Dr. Gunnar Ising for help with the statistical treatment of the material, and to Dr. J. T. Nowakowski, Warsaw, for determination of some of the Cerodontha-species.

To collect material on the island of Öland, I had the advantage of utilizing the Ecological station of Uppsala University situated there.

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LUND 1977 BERLINGSKA BOKTRYCKERIET